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VOL. VI NOVEMBER, 1911 No. 5

THE PHILIPPINE
JOURNAL OF SCIENCE

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PUBLISHED BY

THE BUREAU OF SCIENCE

OF THE

GOVERNMENT OF THE PHILIPPINE ISLANDS

B. MEDICAL SCIENCES

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ADDISON'S DISEASE AND ADRENAL TUBERCULOSIS.

By B. C. CROWELL.

(From the Pathological Laboratory of Bellevue Hospital, New York, and the Biological Laboratory, Bureau of Science, Manila, P. I.)

INTRODUCTION.

Extensive clinical, pathological, and experimental investigations into the pathogenesis of the symptom-complex described by Addison have to a large extent failed to elicit any more positive knowledge of the subject than was originally possessed by that author. Universal recognition has been accorded the establishment of the symptom-complex of asthenia, anæmia, pigmentation of cutaneous and mucous surfaces, with nervous and gastro-intestinal disturbances as a clinical entity, and its frequent association with destruction of the adrenals by tuberculosis. However, the problems of the pathological anatomy and physiology of this disease have become increasingly difficult proportionately to the number of its investigators.

That Addison's disease and tuberculosis of the adrenals are frequently associated and that there is some causal relationship between them is generally admitted; still the occurrence of apparently unequivocal cases of Addison's disease without adrenal destruction, and cases of adrenal destruction by various causes without the appropriate symptoms, has furnished many difficulties in the solution of the problem. In 370 cases of typical Addison's disease collected by Lewin,(1) sound adrenals were found in 12 per cent and diseased ones in 88 per cent.

Typical cases of Addison's disease associated with destruction of the adrenals by causes other than tuberculosis are not infrequent. Some of these have been collected in a recent survey of the literature.

Simple atrophy of the adrenals has been found in Addison's disease by Goldschmidt(2) (one case), Philips(3) (one case), Karakascheff(4) (five cases), Marchand(5) (one case), Bittorf(6) (three cases), Roloff(7) (one case), and Bramwell(8) (one case).

Complete aplasia of the adrenals was reported in one case by Bramwell.(9) *Amyloid degeneration* has also been assigned as the cause in one case by Bittorf.(10)

Tumors of the adrenals in association with Addison's disease have been recorded by Bittorf(10) (two cases, hypernephroma) Marchand,(11) (one case, hypernephroma), and Fleiner(12) (one case, sarcoma).

Thrombosis of the adrenal veins has been described as the cause of acute Addison's disease by Simmonds(13) (one case), Straub(14) (one case), and Bittorf(10) (one case).

Pressure on the splanchnic nerve by an aneurism of the abdominal aorta was reported in one case by Jürgens.(15) This case is of interest as suggesting the splanchnic nerve as bearing a trophic relation to the adrenals. Dreyer,(16) Tschebokearoff,(17) and Asher(18) have proved that the major splanchnic is the secretory nerve of the adrenals and that the vagus has no influence over the adrenal secretion.

Instances of adrenal destruction without Addison's disease are also numerous, and in many of these the adrenals are the seat of tuberculosis.

V. Kahlden(19) has recorded 6 cases of caseous adrenals without Addison's disease.

Wiesel(20) reported a case of a 16-year old girl who had no clinical symptoms of Addison's disease although extensive tuberculosis of the adrenals was present.

Goldschmidt(2) reported 2 cases of tuberculous adrenals without Addison's disease.

Lewin(1) mentioned 44 cases of destruction of the adrenals by various causes in patients showing no evidence of Addison's disease during life and dying of intercurrent affections.

Karakascheff(4) has drawn attention to cases of old hæmorrhage destroying the adrenal medulla without the production of Addison's disease and records an example of this in a child 5 months old.

Although tumors in the adrenal are occasionally associated with Addison's disease, as noted above, yet most frequently they cause no clinical manifestations suggesting it. The reason for this may possibly be due to the infrequency of destructive bilateral tumors.

These cases, which have been selected from a very abundant literature on the subject as typical of the difficulties presented, serve to show the complexity of the problem. Such cases form in part the basis for explanatory theories. However, they are insufficient without a knowledge of the findings outside of the adrenals, which, I think, can best be considered in a general discussion of the subject.

As an introduction to this, a brief sketch of the development of the adrenals is given.

The cortex of the adrenal is developed from the mesodermic epithelium which covers the mesial aspect of the forepart of the Wolffian body, in front of the germinal epithelium. Masses of sympathetic formative cells exist between this primitive adrenal and the aorta, groups of which grow into the adrenal and eventually form the cells of the medulla. These cells increase in size, their nuclei become enlarged and vesiculated, and they acquire the property of staining brown with chromium salts. However, not all of these formative cells enter the adrenal; many remain along the course of the sympathetic nerves in the abdomen and there undergo the same change into chromophil cells. These cells were termed "chromophil cells" by Stilling,(21) "chromaffin cells" by Kohn,(22) and "phäochrome cells" by Poll.(23) Granules or globules of a substance yielding this reaction have been described in the blood within the medullary sinuses and in that of the suprarenal vein. This forms strong histological evidence that the medullary cells pass a secreted substance into the blood.

PATHOGENESIS OF ADDISON'S DISEASE.

A year after his original communication, Addison himself found changes in the abdominal sympathetic in certain cases, and from that time until the present there has been constant discussion as to whether the lesions of the sympathetic system or adrenals are responsible for the symptons. Experiments directed toward the production of Addison's disease by adrenal enucleation succeeded in reproducing all the clinical symptoms except pigmentation, which was not evident during the brief period that the experimental animals lived.

Neusser(24), from a consideration of the cases previously reported and from what was then known of the physiology of the adrenals and sympathetic system, promulgated the following theory: "The suprarenal gland is a gland with an internal secretion. It possesses a double function: first, the neutralization of the toxic products of the metabolic activity of other organs; second, the synthetic production of a substance which is essential to the sympathetic system, maintaining its nutrition and a normal tone. In every case the symptoms of Addison's disease result from impairment, or eventually complete suppression, of these functions of the suprarenal capsules, brought about by disease of the capsules themselves, or of the nerve tracts controlling their functions. These nerve tracts extend from the spinal cord through the splanchnic nerve and the cœliac ganglion. This impairment and eventual suppression of the function of the suprarenal capsules account for the nutritive and functional disturbance of the sympathetic system, on the one hand, and for the general autointoxication, on the other. In addition to these two principal factors, extension of the pathologic process in many cases to the abdominal sympathetic is responsible for the occurrence of some of the symptoms of Addison's disease. Pigmentation of the skin and mucous membranes is not an integral part of Addison's disease, and, though of decided diagnostic significance, is not an essential feature. It is rather an indirect than a direct suprarenal symptom, arising only through the agency of local or general disease of the sympathetic system." The antitoxic property of the adrenal cortex lacks definite proof.

Previous to this time numerous observers had reported the presence of changes in the spinal cord, spinal ganglia, and splanchnic nerves.

It has been known since the time of Henle(25) (1865) that the cells of the adrenal medulla possess a peculiar staining reaction after fixation with chromium salts. Similar cells were also found outside the adrenals especially in the abdominal sympathetic. Kohn(22) created the term "chromaffin bodies" for all such cells in and outside of the adrenals and later conceived the term "paraganglia" for such cell accumulations. The number of these paraganglia, or the places where chromaffin cells are found, has been constantly increasing and the boundaries of the chromaffin system are proportionately extended. Thus, they have been found in the inferior mesenteric ganglia (Zuckerkandl's organ), carotid gland, coccygeal gland, ovary, testis, interventricular septum, aberrant adrenals, and in fact wherever sympathetic nerves are present. The chromaffin of the medullary cells has been found identical with adrenalin, the active substance of the adrenal secretion.

The importance of these findings was emphasized when Wiesel(26) in 1903 propounded the theory that Addison's disease was due to a disease of this chromaffin system and perhaps the adrenal, perhaps the extra-adrenal portions of the system were affected in a given case.

Changes in the sympathetic system had already been described in Addison's disease, and pigmentation of the ganglion cells had been mentioned by Wahneau(27) and Babes and Kalindero(28) among others, but it remained for Wiesel to correlate these findings.

From a careful and minute study of the adrenals and the entire sympathetic system in 5 cases of Addison's disease, all of which had caseous adrenals, the latter investigator propounded his theory. He found changes in the sympathetic nervous system similar to those which had been described by many previous authors, that is, proliferation of the connective tissue of the interstices of nerves, thickening of the walls of the blood vessels, atrophy of the ganglion cells and the formation of pigment and lipochrome in them. However, these he considered as not characteristic of Addison's disease, but rather a purely degenerative process such as occurs with age. He created the term "chromaffin system" to indicate those places where chromaffin cells are found and indicated that this includes the sympathetic nervous system and the adrenal medulla. Addison's disease he conceives as a disease of this chromaffin system which may be attacked at any part either in the sympathetic system or in the adrenal medulla. It consists of a progressive atrophy of chromaffin tissue which, as the chromaffin tissue of the adrenal medulla takes part in this degenerative process, spreads secondarily to the cortex. In order that symptoms of the disease may occur, some considerable portion of the chromaffin system must be affected without regard to the exact location; it is not absolutely necessary for the adrenal gland to be among the diseased structures, and disease of one portion may be compensated for by hypertrophy of others. He does not attempt to say how many of the clinical symptoms are attributable to the involvement of the adrenal cortex and admits that pigmentation of the skin is not accounted for.

Karakascheff's(4) conclusions are in direct antagonism to those of Wiesel.

In 1904 the former reported 2 cases and quoted 3 others with total atrophy of the adrenal cortex and from his findings concluded that the cortex especially is of importance in Addison's disease, and causes the whole symptom-complex. Microscopically, he found the cortex almost completely destroyed and the medulla intact. In his own two cases the cœliac plexus was normal. He reported one case of a child 5 months old with an old hæmorrage into the medulla as evidence that the medulla can be completely destroyed without producing Addison's disease. He also quotes Marchand's(11) case, in which there was extensive disease of the solar plexus and upper cervical ganglion without Addison's disease and 6 cases of v. Kahlden's(19) in which the adrenal medulla was caseous and the cœliac plexus in great part destroyed, without Addison's disease, as evidence that the sympathetic system is not responsible. He also refers to 6 cases of Addisson's disease reported by Simmonds(13) with adrenal destruction, four of which had normal cœliac plexuses.

Bittorf(6) in 1908 rejected Wiesel's theory and championed the pure adrenal theory contending that disease of the sympathetic system can neither cause Addison's disease nor essentially influence the symptoms. Those cases recorded in the literature as clinically certain cases of Addison's disease without adrenal disease he ascribed to inexact anatomical investigation, and those of adrenal destruction without clinical symptoms he would not recognize. He draws attention to the fact, as did v. Neusser, that lack of pigmentation of the skin does not preclude the possibility of Addison's disease and thinks this may account for the failure to make the diagnosis in some cases. According to Bittorf melanoderma and sympathetic changes are the result of adrenal changes, and he mentions the large and richly pigmented adrenals that occur in negros.

Wiesel considers the cortex and the medulla of the adrenal as two separate organs related only topographically, and believes that the function of the cortex is unknown. Bittorf, on the other hand, conceives the mutual physiological interdependence of the two parts and the unity of the organ. According to Bittorf, muscle poisons, and perhaps other similar metabolic products, reach the cortex of the adrenal through its vessels, and there through simple splitting are deprived of their poison; through a further chemical action in the medulla they are converted into the active substance,

So at the present day the two opposite views are held. The "adrenalists" concede that the changes in the chromaffin system may be present but regard them as secondary or accidental. Those who claim the causative lesions are in the chromaffin system admit that the adrenal medulla may be the initial seat of the disease, but only in so far as it constitutes a part of the system does it produce symptoms.

Another factor, the importance of which is as yet unknown, is introduced in the frequently noted association of Addison's disease with *status lymphaticus.* Averbeck(29) noted the condition of lymphatic hyperplasia in his cases of Addison's disease and considered it an essential part of the disease. Star was the first to note persistence of the thymus in Addison's disease, and since then the association of the two diseases has been especially emphasized by Hart,(30) Hedinger,(31) Wiesel,(20) Bittorf,(6) Kahn,(32) and Schridde.(33) Hedinger found status lymphaticus in 7 out of 15 cases of Addison's disease. He considers the possibility of their being due to a common cause which is to be found in an imperfect *Anlage* or in an alteration of the chromaffin system, this latter being suggested by Wiesel's observation of a case of status lymphaticus with marked hypoplasia of the chromaffin system. Kahn suggests the possibility of the lymphoid hyperplasia being an evidence of reaction to the toxins of Addison's disease. Wiesel in his case just mentioned attributed the sudden death to the hypoplasia of the chromaffin system rather than to the status lymphaticus. It is now well known that sudden death is a frequent occurrence in status lymphaticus and that many of these cases have a large thymus gland. These ideas are expressed in the terms "thymus death" and "status thymo-lymphaticus."

Schridde(34) has suggested that death may be due to a kind of endogenous poisoning through excessive or disordered function of the thymus. Warthin(35) especially has considered that the thymus causes death mechanically by tracheal compression, particularly in children. That the thymus in status lymphaticus presents no especial features other than that of delayed resolution or sometimes slight hyperplasia has been shown by Pappenheimer.(36) This delayed resolution brings the weight of the thymus above that of the normal, which latter, within the last few years, has been shown to differ from all previous conceptions. Hammar,(37) Norris,(38) and Pappenheimer(36) have shown that the weight of the thymus increases up to the 15th year and then the gland undergoes involution. Delayed involution occurs from several causes.

Disease of other glands of internal secretion is not infrequently associated with status lymphaticus, the most noteworthy instance being Basedow's disease. In fact a hypoplasia of the genital organs is claimed by some as an almost constant feature of status lymphaticus.

The insufficiency of our knowledge of Addison's disease is very apparent from a consideration of the conflicting theories just discussed. Hence, the following cases are reported with the idea of furnishing data which may be of value in arriving at its correct interpretation.

REPORT OF CASES.

The present writer had the opportunity of studying two cases of Addison's disease during his service in the Pathological Laboratory of Bellevue Hospital, and has recently encountered two instances of adrenal tuberculosis in Filipinos in Manila.

The first case, number 2142, was that of an Italian married woman, 39 years of age, a shopworker, who was under observation only 48 hours before death. The history was that of good health until 3 months previous to admission to the hospital. She then had severe pain in the abdomen and back, which for a time became less severe, and was accompanied by insomnia and loss of appetite and strength. After 2 months her weakness became so marked as to confine her to bed, and she suffered from abdominal pain, vomiting, hiccough, insomnia, constipation, and headache. During the last 3 months of her life she lost 30 pounds in weight and her face and hands grew darker during the last month.

While under observation the essential clinical features were pigmentation (described below), marked asthenia, weak pulse (84 millimeters of mercury), and signs of apical tuberculosis. There occurred an ante-mortem rise of temperature to 104°.2 F. (40°.1 C.) and an attack of pulmonary œdema ended fatally.

The autopsy, which was performed 13 hours after death, was limited to an abdominal incision, through which, however, it was possible to remove the thoracic viscera.

PROTOCOL 2142.

The body is that of a well-formed, fairly well-nourished white female of short stature, 39 years of age. Rigor mortis is marked, and there is considerable post-mortem suggillation over the dependent parts. There is no œdema of the skin. The face and back of the hands, the lips, and the gums show brownish pigmentation. The chest is well developed and well formed. The nipples and areolæ are extremely small and black. The waist is narrow. The thighs are well arched laterally, the arching starting from the waist line; anterior arching is not marked. Thighs and legs have a few long hairs. The pubic hair is well developed. There is abundant lanugo over the abdomen. There are hairs over the upper arms, moderate in amount. The hair of the head is abundant and dark. The eyebrows are well developed. There is hair on the nose between the eyebrows, a fairly well developed moustache and long hairs on the chin. There is a moderate amount of golden-yellow subcutaneous fat. The peritoneum is slightly thickened. The great omentum is long and thin, with a small amount of fat and is adherent to the dome of the bladder and to the anterior abdominal wall. The intestines are collapsed; the serosa is clear, pale, and glistening. The mesentery contains a moderate amount of fat, with a few very pale lymph nodes about 1 centimeter in diameter. The liver reaches the right costal margin and is firmly adherent to the diaphragm. The diaphragm reaches 4th interspace on the right and 5th on the left.

Lungs.—There is no fluid in the pleural sacs. The lungs are firmly adherent to the parietes throughout, and each shows fibrocaseous areas near the apex, the remaining lung substance being crepitant and soft, with a smooth surface. The pleurae show a few punctate hæmorrhages. Bronchi and vessels are in good condition. The lymph nodes at the hilus are enlarged and anthracotic, and one or two show fibrous foci.

Thymus.—Not discovered owing to the method of section.

Thyroid.—The thyroid is slightly enlarged, weighing 35 grams. On section it is more pale than normal, but shows definite glandular structure, apparently with a small amount of colloid.

Heart.—The pericardium is firmly adherent to the heart, which is small, without hypertrophy of either ventricle. Endocardium is intact, except for slight pallor and thickening of the mitral valve. The heart muscle is very brown and rather soft. The coronary arteries are in good condition. The aorta shows some fatty degeneration of the intima, especially about the isthmus.

Spleen.—The spleen is of about normal size, weighing 150 grams. It is of good consistence and the capsule is thin and smooth. The cut surface is smooth and dark red. The Malpighian bodies are easily visible.

Adrenals.—The left adrenal is small and very firm, and on section none of the normal markings of the gland can be distinguished, the gland substance being completely replaced by large, white, partly caseous, masses.

The right adrenal is considerably enlarged, its surface being pale and nodular. On section there are seen few of the markings of the gland, the cortex being represented by faint yellowish streaks. At the lower pole is a large caseous focus, and surrounding the entire cortex, for a width greater than that of the normal gland, is a pale, firm tissue containing a few caseous foci.

Kidneys.—The kidneys are of normal size with thin capsules which strip easily from smooth pale surfaces. The two kidneys together weigh 275 grams. On section the consistence is diminished, the cortex is broad and pale, with somewhat injected Malpighian bodies. The pyramids are of a deep blue color. The pelves, ureters, and urinary bladder are free. *Uterus.*—The uterus is small, firm and pale. The endometrium is intact. A mucous plug extrudes from external os. *The Vagina.*—Is normal. *The Fallopian tubes.*—Both are considerably distorted, folded on themselves and adherent, the left being adherent to the anterior wall of the uterus. They are also adherent to the *ovaries*, which are small, firm, pale, and sclerotic, with a few small cysts. Hanging on the broad ligament at the fimbriated extremity on each side are small unilocular cysts containing a dark fluid.

Stomach and intestines.—Contain a considerable amount of pea-green, fluid material, but no formed fæces. Duodenum and stomach show, on their mucous surfaces, numerous rather closely packed but still discrete small pale elevations.

Liver and gall bladder.—The liver weighs 1,500 grams and is slightly diminished in size. The capsule is somewhat thickened where adherent to the diaphragm. On section the consistence is somewhat diminished, and the cut surface is dark brown in color, showing the lobules but indistinctly. *Bile ducts. Normal,* *Gall bladder.*—Contains a considerable amount of dark bile with yellowish sand-like matter suspended in it. Its mucosa is apparently normal.

Pancreas.—The duct of Wirsung is normal. The pancreas is small, narrow, flat and pale, and weighs 75 grams. There are numerous enlarged and caseous retroperitoneal lymph nodes.

The gross anatomical diagnosis was: Chronic fibro-caseous tuberculosis of both adrenals; melanoderma; chronic adhesive pleurisy and pericarditis; healed apical pulmonary tuberculosis; healed tuberculosis of bronchial and retroperitoneal lymph nodes; lymphoid hyperplasia of stomach and intestines; chronic parenchymatous nephritis; chronic salpingitis and oöphoritis; status lymphaticus. No accessory adrenals were found. The thymus could not be isolated on account of the method of section.

Microscopically the adrenals showed complete destruction, being entirely replaced by a central caseous area surrounded by tuberculous granulation tissue containing some miliary tubercles and areas filled with plasma cells. The thyroid showed microscopic tubercles as did the liver and spleen. The pancreas showed a marked hypertrophy of the islands of Langerhans. The semilunar ganglia, which were the only parts of the sympathetic system preserved, contained chromaffin.

The investigation of the chromaffin system in this case was limited to the adrenals and semilunar ganglia, in the former of which it was entirely lacking. Thus far this case might well accord with either Wiesel's or Karakascheff's theory and forms alone an insufficient field for theorizing as to the pathogenesis of the disease. However, the simultaneous lesions in the adrenals, thyroid, and pancreas, form apparent histological evidence of what has been shown experimentally in other connections. Eppinger, Falta, and Rudinger(39) have shown that both adrenals and thyroid inhibit the pancreas, and Falta(40) has reported one case in which after thyroidectomy an undoubted hypertrophy of the islands of Langerhans occurred. In our case there was complete suppression of adrenal function and in all probability dysthyroidism can be postulated in view of the thyroid tuberculosis. This would remove normal inhibition from the pancreas and lead to the hypertrophy of the islands of Langerhans, which is supposedly histological evidence of hyperfunction.

The second case, number 2262, was that of a Canadian farmer, 34 years of age, who at 3 years of age had a lesion of the left hip joint which resulted in complete ankylosis. He had had bronzing of the skin for 11 years, and had lost 25 pounds in weight in the 3 months previous to his entrance to the hospital, where he sought admission on account of weakness, soreness of the muscles, and loss of appetite. Aside from his ankylosed hip, pigmentation, asthenia, soreness of the muscles and low blood pressure (85 millimeters of mercury) he presented no noteworthy clinical symptoms.

PROTOCOL 2262.

The body is that of a well built and muscular adult white male of about 35 years. The left lower extremity is 6 to 8 centimeters shorter than the right and is flexed and adducted. Rigor mortis persists, but is disappearing in toes and fingers. The left leg can not be rotated, apparently being fixed at the hip, and is considerably less in circumference than the right at both thigh and calf. The skin of the head, especially that of the forehead, is a very dark brown, resembling sunburn, this being less marked over the face and neck. The skin of the trunk and abdomen is dirty yellowish in color. The dorsum of both hands is deep brown in tint, as if sunburnt. Over both extremities and trunk in front and behind are numerous symmetrically distributed lesions, all being about 7 millimeters in diameter, round, macular, with a narrow, dark brown pigmented rim, and a whitish, smooth central portion, not elevated above the surface and not scaly. In a few of the maculæ the central portions are slightly depressed. The inner portions of the lips and mucous membranes of the mouth show a few black pigmented areas. On the skin of the penis and glans are several dark bluish pigmented areas of small size (2 or 3 millimeters).

The hair of the scalp is of good growth, brownish, lustrous. The beard and moustache are scant. The axillary hair is scant, the individual hairs long. There are a few long black hairs around the nipples, scant short hair on the trunk, a few also on lineæ of abdomen, and moderate pubæ. The hair on the thighs and lower legs is poorly developed. The anus and perineum have a good growth of hair. There are no lesions around the anus. No mucous patches are visible.

There is no œdema of the subcutaneous tissues.

The head is moderately round in type. The pubic hair is feminine in type. The superficial lymph nodes are not palpable.

On section there is a small amount of panniculus abdominis. The musculature is everywhere well developed. The abdominal cavity is free from fluid and adhesions. The liver reaches the costal margin. The diaphragm is at 4th rib on the right and 4th space on the left. The costal cartilages are not ossified.

Lungs.—The left lung is everywhere adherent; the right everywhere free. Right pleura is filled with a clear straw-colored fluid. The left lung is everywhere well aërated and dry; it is slightly congested. The right lung, except for apical thickening of the pleura, resembles the left. The bronchial lymph nodes are small and anthracotic. There are no signs of tuberculosis in the nodes or in the lungs. The bronchi are congested, otherwise normal.

Thymus.—Persistent. It is red and fleshy, and in places well developed. It extends to the auricle over the pericardium where it is thin. It weighs 21.5 grams.

Heart.—The pericardial sac is not distended; it contains 20 cubic centimeters of clear fluid. The epicardial fat is moderate in amount, being excessive at the apex. There is no distension of the cavities, which contain chicken-fat clot, especially on the right side. All the valves are normal except the mitral which is thickened at the margins. The anterior papillary muscles of the mitral are short and thick at the apex; chordæ tendinæ are short. The apices of the papillary muscles are pale and thickened. The aorta is normal as to thickness and elasticity, the abdominal portion shows above the bifurcation a whitish, raised patch. The iliacs normal. The aorta measures 75 millimeters above the cusps, 45 millimeters at the cœliac axis, and 35 millimeters at its bifurcation.

Spleen.—Normal in size and appearance.

Adrenals.—The left adrenal measures 55 by 22 by 12 millimeters and weighs 6.5 grams. It retains its original contour. On section it is seen to be apparently completely replaced by a pale yellowish caseous mass, and there is no vestige of glandular tissue visible. At the anterior extremity the vessels of the medulla can be seen. The right measures 65 by 30 by 13 millimeters and weighs 12 grams. It also retains its contour and is replaced by a yellowish caseous material, which extends to the capsule. At its lower and posterior extremity the medullary vessels are visible with perhaps a small amount of cortical substance at the periphery.

Semilunar ganglia.—On the two sides considerably larger than normal, but very flat and rather firm in consistence.

Kidneys.—The kidneys are embedded in a small amount of perinephric fat, and are of about normal size with thin capsules which strip easily from pale surfaces. On section they are of diminished consistence (owing to post-mortem changes). The cortex is of normal width, rather pale with irregular red striations. The pyramids are also rather pale. The pelves and ureters are free.

Urinary bladder.—Contracted and empty. A few ecchymoses on the posterior wall.

Intestines.—The lower half of the small intestine shows many raised hypertrophic lymphoid follicles. All the Peyer's patches are raised, and hyperplastic, and are occupied by a follicular, stippled mass of tissue. Some of the Peyer's patches show mottled greenish and black pigmentation. The large intestine in its lower portion contains a fairly solid mass of fæces. The follicles are everywhere numerous, prominent and flat, with pigmented borders. The mesentery contains a moderate amount of fat.

Stomach.—Normal in size, without distension. Its mucosa is clean and covered with mucus. No lymphoid hyperplasia is visible.

Liver.—Normal in size. The capsule is smooth. The organ is dark brown in color. Section shows a normal appearance with rather indistinct markings. The gall bladder is normal, and slightly distended. The portal vessels are normal. The bile duct is patent.

Pancreas.—Pinkish in color. Normal in size and lobulations. It measures 160 millimeters in length, 12 millimeters in average thickness, the head being 45 millimeters wide and the tail 30 millimeters and weighs 65 grams.

Neck organs.—Tongue is large, papillæ at base being prominent. The lingual tonsils are very large. The pharyngeal tonsils are enlarged irregularly and indurated. The lymphoid tissue of the pharynx and larynx is hypertrophic. There is a slight laryngeal œdema. The trachea is normal. The œsophagus in its lower portion shows several raised, whitish, linear patches.

Thyroid.—Rather large. On section it shows normal colloid appearance. It weighs 45 grams, each lateral lobe being 50 by 30 by 20 millimeters and the isthmus proportionate in size.

The parathyroids are small.

Lymph nodes.—The cervical and axillary lymph nodes are small and pale. The mesenteric lymph nodes are very small and pale, except for one rather large one (about 1 centimeter in diameter). There are some flat nodes in the ileo-cæcal chain.

Brain.—The skull and scalp are normal. The dura is normal. The pia shows no œdema. The vessels at base are normal. The ventricles and ependyma are normal. Section of brain substance reveals no gross lesion. Ears normal. The pituitary is normal in size.

After excision of the hip joint, a transverse section through the trochanter and head of the femur and the acetabulum shows the neck of the femur considerably shortened, and the head of the bone firmly fixed in the acetabular cavity, so that the outline of the margin between the femur and the acetabulum is very indistinct. About this line there is a considerable amount of firm, white, bone tissue. The marrow of the head shows one area of complete destruction about 2 centimeters in diameter. The marrow is replaced by fibrous tissue in some places, and in others is destroyed and replaced by a yellowish-white substance which is softer than the bone.

The gross anatomical diagnosis was: Caseous tuberculosis of the adrenal glands; chronic coxitis (tuberculous?); chronic adhesive pleurisy; chronic ossifying myositis of left gluteus medius; hyperplasia of lymphoid tissue of lingual and faucial tonsils, pharynx, larynx, spleen, small and large intestine; congestion of lungs; status lymphaticus. No accessory adrenals were found and there was no arterial hypoplasia.

Microscopically, the adrenals showed the same complete fibro-caseous destruction with only small vestiges of the cortex at one part. The semilunar ganglia contained chromaffin. There were some small cervical nodes which contained healed tuberculous areas. The pancreas and thyroid showed no marked pathological changes. One parathyroid which was sectioned was found to be the seat of a very extensive fibrosis, showing only small remnants of the gland embedded in the fibrous mass. The thymus gland showed marked hyperplasia.

Extracts of the adrenal masses were made according to the method described by Wells and Greer,(41) and the intravenous injection of 2 cubic centimeters produced no rise of blood pressure in the rabbit. The extract of a normal adrenal, obtained the same length of time after death and prepared in exactly the same way, produced a distinct, sharp rise when 0.5 cubic centimeter was injected. In order to eliminate a possible counteracting depressor action of any of the products of caseation, an extract of caseous mesenteric glands from a tuberculous infant was injected intravenously and no change in blood pressure was observed.[1]

Similarly, the extract of the caseous adrenals gave a negative Ehrmann's reaction; that is, it produced no dilatation of an excised frog's eye in several hours, while the extract of the normal adrenal produced temporary dilatation, as did also adrenalin chloride in 1 in 10,000 solution. These tests were performed with naked-eye readings simultaneously under identical conditions of temperature and light, and the independent readings of four observers coincided. This is mentioned because Schultze(42) has devised an elaborate apparatus for observing the pupil and making accurate measurements under constant light and temperature and considers naked-eye readings of no value. Oliver and Schaeffer(43) who were the first to describe the angiotonic effect of adrenal extract on intravenous injection, have also found the extracts of the adrenals in two cases of Addison's disease inactive as far as the blood-pressure-raising reaction was concerned.

The salient features of this second case, then, were prolonged bronzing of the skin, with acute symptoms of Addison's disease lasting 3 months, adrenal tuberculosis without other tuberculous foci except those long healed, parathyroid fibrosis, and status lymphaticus. It is worthy of note that adrenal tuberculosis may occur without other active or recognizable tuberculous foci. It is also true that severe loss of weight may occur on account of adrenal tuberculosis without other widespread tuberculous lesions. This patient lost 25 pounds in 3 months. This case, like the first, showed a status lymphaticus and destruction of another endosecretory gland.

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[1] Since writing this there has come to hand an article by Clyde Brooks in *Journ. Exp. Med.* (1911), 14, 550, on the Absence of Adrenalin in Malignant Renal Hypernephromas. In this he reports negative physiological tests for adrenalin in (A) Metastatic tissue from an adrenal tumour,

(B) Two typical hypernephromas,

(C) Blood from patient with malignant hypernephroma. In his report of the literature he omitted the report of my tests which was published in *Trans. N. Y. Path. Soc.* (1909), N. S. 9, 80.

The two cases of tuberculosis of the adrenals which the writer has had the opportunity of studying in Manila were accidental autopsy findings in which the clinical diagnosis was generalized tuberculosis. Both of these occurred in Filipinos, thus raising the question as to the possibility of making the clinical diagnosis of Addison's disease in dark races. Bittorf and Neusser state that melanoderma is not a necessary integral part of Addison's disease, but agree that it is of very great diagnostic importance. In these cases no excessive pigmentation of the mucous membranes or of the skin could be observed on very close examination on the autopsy table, aside from two isolated dark spots on the tip of the tongue of the second case. It was considered at the time that these might have occurred from extraneous sources, such as the teeth, and microscopical examination was of little assistance, since the pigment involved not only the epithelium but the corium as well, and might have reached the part from either direction.

The first case, number 1350, was a male Filipino, 26 years of age, who had been imprisoned for 7 years and had been in the tuberculosis ward of the hospital for 3 years. From May, 1906, until April 15, 1908, when he was transferred to the tuberculosis ward, various diagnoses were made at different times and these diagnoses constitute the only available record during that period. These were, acute dyspepsia, acute articular rheumatism, malaise, acute bronchitis, neurosis (anæsthesia of the right arm), intestinal colic and conjunctivitis. In 1907 amœbæ and ova of *Trichuris trichiura* were found in his fæces, but disappeared after treatment. The urine was negative. Tubercle bacilli were found in the sputum for the first time in April, 1908. *At autopsy* he was found to be a much emaciated Filipino who showed no excessive pigmentation of his mucous membranes. The skin being normally of a uniform, dark color, no pathological bronzing could be determined. The anatomical diagnosis made at the autopsy was: Chronic ulcerative pulmonary tuberculosis (very extensive); chronic adhesive pleurisy, tuberculous; chronic bronchitis; tuberculous colitis; congestion and parenchymatous degeneration of the liver; parenchymatous degeneration of the heart and kidneys; tuberculous lymphadenitis of the bronchial and cervical nodes; tuberculosis of adrenals.

The pulmonary tuberculosis was very extensive, practically all lung tissue being destroyed.

The left adrenal weighed 5 grams and it showed on the surface smaller and larger foci, the largest measuring as much as 1 centimeter in diameter. On section these foci were firm, pale, circumscribed, and occurred mostly in the cortex of the gland, although some extended through the cortex to the medulla; otherwise the adrenal tissue had a normal appearance. The right adrenal weighed 4.5

grams and presented a similar appearance. The spleen weighed 210 grams, pancreas 90 grams, thyroid 9.3 grams, and the testes 9.5 and 10 grams respectively. The spleen showed some interstitial splenitis, but the rest of these organs the weight of which is given showed no change. The weights of these viscera form about the average of the weights of the organs in the tuberculous cases without adrenal involvement.

There was no evidence of status lymphaticus in this case and the thymus was embedded in such a mass of mediastinal adhesions that it was not isolated. The miscroscopical examination of the adrenals confirmed the gross anatomical diagnosis. Extensive caseation of the cortex of the adrenal occasionally involving small parts of the medulla constitutes the main lesion. The caseous areas are surrounded by zones of small round cells *without* fibrosis. This lack of reactive connective tissue is quite uniform throughout this case, and indeed appears characteristic of the tuberculous lesions as encountered in Filipinos. The cortex is not all destroyed, and that which remains shows a nodular hyperplasia and dilatation of the capillaries. The individual cells, except where compressed by the tubercles, show the normal structure; the protoplasm stains rather poorly with eosin and is reticulated; the nuclei contain abundant chromatin and occasionally assume giant forms. The inner zone of the cortex contains abundant native pigment (not chromaffin). The medulla shows areas of coagulation necrosis even where not directly the seat of tuberculosis, with pyknosis of the nuclei and even complete nuclear destruction. Chromaffin is very scant. The semilunar ganglia contain chromaffin.

The second case, number 1352, is that of a 57-year old Filipino who had been in prison 2.5 years. In 1909, two years before his death, he had a "tuberculous abscess of the scrotum." One week before death he was admitted to the hospital on account of general weakness. His weakness was extreme and for 2 days previous to death he was practically pulseless. Aside from the evidence of the old scrotal abscess, there were but few symptoms on which to base a diagnosis.

Dr. J. W. Smith, under whose charge the patient was, made the diagnosis of generalized tuberculosis in spite of the lack of definite physical signs and the absence of tubercle bacilli from the sputum.

At autopsy the only evidence of excessive pigmentation consisted in 2 small, dark areas on the sides of the tongue. He was extensively emaciated. The gross anatomical diagnosis was: Chronic miliary pulmonary tuberculosis; emphyseoma of the lungs; chronic adhesive pleurisy; tuberculous enteritis; parenchymatous degeneration of the heart, liver, and kidneys; chronic perisplenitis; tuberculosis of both adrenals, the right kidney, prostate gland, left epididymis, right testicle, and spermatic cords.

The left adrenal measures 6 by 1.5 by 1 centimeter and weighs 4.1 grams. It is pale and on section presents numerous smaller and larger conglomerate caseous foci. The normal architecture of the gland is completely obliterated so that no adrenal tissue can be recognized. The right adrenal measures 5.5 by 3 by 2 centimeters and weighs 10.7 grams. This is also distorted in shape and the seat of one large nodule (2 by 2 by 1.5 centimeters) and several smaller ones, so that only a small vestige of the gland can be recognized situated at the lower pole. These nodules are pale, fairly firm, with yellow striæ traversing them. The spleen weighs 120 grams, testes 9.3 and 8.2 grams, pancreas 54 grams, and thyroid 7.3 grams. The weights of adrenals and testes are increased on account of tuberculosis. Microscopically, no vestige of medullary tissue is found in the adrenals. There are islands of intact cortical cells and many islands of these cells surrounded by and included in the tuberculous tissue in all stages of degeneration and necrosis. The capsule is thickened and there is a small amount of granulation tissue beneath it which includes many vessels which are the seat of a very marked endarteritis and periarteritis. The proliferated adventitia of the vessels frequently contain numerous tuberculous giant cells. Plasma cells are numerous. Chromaffin tissue is present in the cells of the semilunar ganglia in small amount.

These two cases occurred in the hospital at Bilibid Prison where there is a large number of patients under the care of one physician, and these cases did not attract enough attention to make them the subject of any elaborate physical examination.

The writer is inclined to believe that the first case was a simple case of adrenal tuberculosis without Addison's disease. However, the second case is more probably one of Addison's disease. The extreme prostration of the patient and the low blood pressure during the last week might possibly have been accounted for by his widespread tuberculosis, but the fact that he was able to be at work until a week before his death would seem to point to some more definite anatomical feature which could account for a lack of some necessary tonic secretion. In this case especially the anatomical evidence is that the adrenals had been functionless for a considerable time and the final prostration would more plausibly be accounted for by the eventual failure of other tissues to carry on the vicarious function of the adrenal. This appears to me to be a very strong point in favor of viewing the adrenals as one part of a system, the complete destruction of which may for a time be compensated for by other parts of the system. Whether this system be the adrenal medulla and the sympathetic nervous system, or the adrenal cortex and some other gland which may act vicariously for it, is not proved by my experience.

SUMMARY.

1. Two clinically certain cases of Addison's diseases have been described in which the adrenals were destroyed by tuberculosis and the semilunar ganglia contained chromaffin. In both of these, other endosecretory glands were the seat of pathologic processes. In one, hyperplasia of the islands of Langerhans was associated with adrenal and thyroid tuberculosis; in the other, one parathyroid was cirrhotic.
2. A case of adrenal tuberculosis without changes in the semilunar ganglia and without recognized Addison's disease occurred in a Filipino.
3. A second case in a Filipino with adrenal tuberculosis and intact semilunar ganglia was probably one of Addison's disease.

The difficulty of diagnosing Addison's disease in dark-skinned races is great on account of the absence of pathological melanoderma and the possible presence of wide spread tuberculosis. If Addison's disease does occur in dark-skinned races it substantiates the opinion that melanoderma is not an essential feature of the disease, while it emphasizes its importance as a diagnostic sign.

4. The adrenals from one case of Addison's disease contained no adrenalin estimable by the angiotonic and pupil-dilating reactions.

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RICE AS FOOD: INVESTIGATION OF THE NITROGEN AND PHOSPHORUS METABOLISM ON A DIET CONSISTING PRINCIPALLY OF RICE AND OTHER VEGETABLE FOODSTUFFS.

By Hans Aron and Felix Hocson.

(From the Department of Physiology, College of Medicine and Surgery, University of the Philippines.)

INTRODUCTION.

Rice is probably the most prominent vegetable food of man. A rough estimate shows that in Asia alone at least 400 million Indians, Chinese, Japanese, and Malays consume rice. Only a small quantity of other food is eaten by the lower classes of these people, because their low earning power does not permit a richer diet. The question has been considered frequently whether such a diet is a sufficient one, and it gains further importance because of the close connection which has been proved to exist between the consumption of certain classes of rice and the existence of those polyneuritic diseases which are commonly termed beriberi.

The variations in the composition of the different varieties of rice are not so great but that from our standpoint we can speak of rice in general. However, the different methods of treatment and milling alter considerably the composition of the grain, as has been pointed out in a previous paper.[1]

We have determined the phosphorus content of 28 different classes of rice during the past two years. The following table shows plainly that the phosphorus content within narrow limits is determined by the grade of milling. The whiter the rice the poorer it is in phosphorus.

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[1] *This Journal, Sec. B* (1910), 5, 81–98.

TABLE I.—*Composition of various classes of rice milled in different ways.*

Number of sample.	Variety of rice.	Appearance.	P_2O_5.	Nitrogen.	H_2O.
			Per cent.	*Per cent.*	*Per cent.*
1	Liverpool	Very white	0.15	1.07	11.67
2	Valenciana	do	0.20	--------	--------
3	Metabolism experiment, May, 1910.	Pure white. Trade-mark "Ia, Blanco."	0.28	1.29	11.75
4	Metabolism experiment, January, 1910.		0.28	1.32	--------
5	Laguna, Ia Blanco		0.31	--------	--------
6	Ilocano "Sup. Blanco"		0.32	1.20	--------
7	Metabolism experiment, 4 ff		0.32	1.06	--------
8	Culion, December, 1908		0.32	--------	--------
9	Manila Market		0.33	--------	--------
10	Metabolism experiment, January, 1910.	White	0.33	1.48	--------
11	Macán machinery rice		0.34	--------	--------
12	Metabolism experiment, 5	Quite white slightly yellowish.	0.37	1.23	--------
13	Bombay	Brownish, pericarp incompletely removed.	0.41	1.25	11.97
14	Cape Engaño	Slightly brownish, pericarp partly removed.	0.43	--------	--------
15	Tagalog D	Slightly brownish, pericarp partly removed.	0.45	1.42	--------
16	Calcutta	Brownish, pericarp incompletely removed.	0.45	1.24	11.63
17	Culion, January, 1910		0.46	--------	--------
18	Macán, Native Mill		0.46	--------	--------
19	Siam, Asylum	Brownish, greater part of pericarp not removed.	0.52	--------	--------
20	Tagalog C		0.53	1.43	--------
21	Laguna, slightly milled		0.56	--------	--------
22	Metabolism experiment, January, 1910.		0.57	1.19	--------
23	Tonkin		0.58	--------	--------
24	Ilocano 4		0.58	--------	--------
25	Culion, October, 1910		0.60	1.19	--------
26	Tagalog Pinawa	Husked only and cleaned of husk by air blast.	0.73	1.54	--------
27	Ilocano Pinawa		0.75	1.27	--------
28	Pinawa, Metabolism experiment, 3.		0.77	1.29	--------

TABLE II.—*Descriptions of rice samples.*

Sample number.		Treatment.	Phosphoric acid anhydride.	Protein. (nitrogen x 66.25 per cent.)	Weight in 1.000 grams.	Removed by milling.
			Per cent.			*Per cent.*
Ilocano rice.	1	Husked only, "Pinawa"	0.77	7.94	23.69	--------
	3	Larger distance between cones than is usual: undermilled	0.58	--------	22.55	4.8
	5	Superior Blanco; overmilled	0.32	7.50	20.52	13.4
Tagalog rice.	A	Husked only, "Pinawa"	0.73	9.63	--------	--------
	C	One polisher, one cone, regular distance; IIa Blanco	0.53	8.96	--------	--------
	D	Two polishers, two cones regular distance, grains partially broken	0.44	8.90	--------	--------

The nitrogen content of rice differs considerably less than the phosphorus content, but the more intensively a given rice is milled the poorer it becomes in nitrogen; however, the nitrogen content of different classes of rice varies so greatly that this figure, in any particular case, gives no indication of what treatment the grain had been subjected to in milling. For practical purposes, we can distinguish three stages of milling if we judge by the phosphorus content of the rice. These are: (1), rice, husked only, 0.7–0.8 per cent phosphoric anhydride; (2), undermilled rice, 0.45 to 0.6 per cent phosphoric anhydride; (3), overmilled rice, 0.15 to 0.35 per cent phosphoric anhydride.

Rice as food, has the following characteristics: (1), it is comparatively poor in protein; (2), it is very rich in carbohydrates, especially in starch; (3), the white variety is low in ash and especially in phosphorus. One hundred grams of rice, representing about 350 calories, contain only 7 to 8 grams of protein. Therefore, the daily demand for energy of some 2,100 to 2,400 calories would be supplied by 600 to 700 grams of rice; on the other hand, this quantity would contain only 45 to 55 grams of protein; and if the grain were white, it would then afford only 1.5 to 2 grams of phosphoric anhydride. In fact, the quantities of the latter constituent which are taken will often be still lower if other foods, rich in carbohydrates or fat, are consumed in addition.

Several authors point out the fact that a diet consisting for the greater part of rice must of necessity be very voluminous. This idea is based on the supposition that the rice-eating native cooks his rice in the same way as the European. Scheube[2] has shown this to be erroneous. Our own observations as well as those of other residents in the Orient prove that the Japanese, Chinese, and Malays cook rice with so little water that, although the grain becomes softened, it remains apparently dry. In carrying on metabolism experiments, we have weighed, twice daily for ten days, rice prepared by natives. This was done before and after cooking, and we found that within narrow limits 100 grams of uncooked rice gives about 250 grams of cooked rice. Rarely is more than 300 grams of rice, which cooked would weigh 750 grams, taken at one meal. This is not a very great amount, especially when we consider that the Malay at least, and, we believe, the Chinese and Japanese also, seldom drink with their meals; the majority of them drink afterward, and even then only small quantities. An average European very frequently drinks as much as one liter of liquid with each meal, so that his meal certainly is a much more voluminous one than that of the rice-eating Oriental.

These considerations induced us to investigate the nitrogen and phosphorus metabolism of people living mainly on rice or on a similar diet more or less deficient in nitrogen and phosphorus. As these questions seemed to us to be of fundamental importance, and as they were emphasized by Schaumann[3] in his last extensive publication, we continued the experiments which were described previously,[4] using prisoners in Bilibid Prison. Our thanks are again due to the authorities of that institution for their kindness. Two medical students[5] also served with great enthusiasm as subjects for further experiments.

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[2] *Arch. f. Hyg.* (1883) 1, 352–83.

The basis of all rations was rice. In addition, we gave varying amounts of vegetable and animal foodstuffs, such as bread, fruits, vegetables, sugar, fish, bacon, and lard. The bread used in a great number of experiments was made of fine wheat-flour, very similar to white rice in its content of nitrogen, phosphorus, and carbohydrates. The arrangement of the experiments was practically the same as described in our previous paper. The subjects were all healthy men. Most of the prisoners had previously undergone small surgical operations, but had fully recovered at the time they volunteered for the experiments. We paid especial attention to securing men free from intestinal disturbances and who had good digestion. Their fæces, in two microscopical examinations, were free from intestinal parasites and eggs. The prisoners were isolated in a shady room of the hospital and did no work during the experiments. An exact control was possible only in this way. At the same time the secretion of sweat could be reduced to a minimum. Both medical students who took part in the experiments lived near the laboratory and performed but little laboratory work during the period of special diet. Therefore, the daily amount of work which our subjects did was extremely limited and the food was comparatively low in caloric value. Experiments conducted during severe muscular work with a corresponding greater intake of food of composition similar to that given in our experiments, are desirable. However, such experiments in the Tropics would be very complicated owing to the necessity of collecting the sweat and determining the nitrogen secreted in it.

Four experiments[6] not open to criticism, have been found by us in the literature. In these, nitrogen metabolism has been determined on a diet consisting mainly of rice. They are compiled in Table III.

So far as we know, phosphorus metabolism on a diet such as that given in Table III hitherto has not been investigated. A summary of our own 15 experiments on 9 normal men is given in Table IV. It shows the quantities of nitrogen and phosphorus taken with the food, the amounts of nitrogen and phosphorus excreted in the urine and fæces, the percentage distribution of nitrogen and phosphorus, and, finally, the nitrogen and phosphorus balance.

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[3] *Arch. f. Schiffs- u. Trop.-Hyg., Beiheft* (1910), **14**, 13–372.

[4] *Loc. cit.*

[5] Messrs. Velarde and Feliciano.

[6] Max Rübner, *Ztschr. f. Biol.* (1879), **15**, 115–202; Karl Thomas, *Arch. f. Anat. u. Phys.* (1909), 219–302; Muneo Kumagawa, *Virchow's Arch.* (1889), **116**, 370–431.

TABLE III.

Number.	Author.	Subject.	Body weight.	Daily food.	Duration of experiment.	Calories.		Nitrogen—								Nitrogen balance.
								Intake—				Out put—				
												Urine.		Fæces.		
						Total.	Per kilogram body weight.	In rice.	In other food.	Total.	Per kilogram body weight.	Grams.	Per cent of intake.	Grams.	Per cent of intake.	
			Kilos.		*Days.*			*Grams.*	*Grams.*	*Grams.*	*Grams.*					*Grams.*
1	Rübner (4)	Man 23 years old.	72	638 grams rice 24 grams meat extract 82 grams bone marrow	2	2,970	41	8.9	1.55	10.45	0.14	11.59	<100	2.13	20.4	−3.27
2	Thomas (5)	Author	75.9	500 grams rice flour 320 grams milk sugar 100 grams starch 30 grams cane sugar 2 lemons and salt	2	3,615	47	5.04	-------	5.04	0.07	4.52	89.9	1.64	32.6	−1.12
3	Kumagawa (6).	Author; 27 years old.	48	450 grams rice 80 grams miso 250 grams turnips 10 grams shoyu 7 liters tea 800 cubic centimeters beer	5	2,030	42	4.46	2.61	7.07	0.15	7.06	99.9	1.66	23.5	−1.65
4		Japanese	48	600 grams rice 100 grams miso 300 grams turnips 10 grams shoyu 585 cubic centimeters tea 595 cubic centimeters beer	9	2,590	54	6.00	2.76	8.76	0.19	6.07	6.93	2.03	23.2	−0.66

TABLE IV.

Experiment number.	Subject.	Body weight.	Daily intake of food.							Duration of experiments.	Calories.	
			Rice.	Bread.	Fish.	Sugar.	Bacon.	Coffee.	Other food substances.		Daily.	Per kilogram of body weight.
		Kilos.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*		*Days.*		
1	Medical student V	35.7	400			45	60	150	100 grams bananas 200 grams rice cake	3	2,360	45
2	Medical student F	54.0	400			40	60	175	150 grams bananas 200 grams rice cake	3	2,400	44
3	Prisoner 20560 (C.)	49.0	[a]400			50	50	300	100 grams onions 20 grams lard	4	2,200	44
4	do	48.6	[b]400			50	50	300	100 grams onions 20 grams lard	4	2,200	45
5	Prisoner B	64.0	300	400		100	75	200		4	2,600	41
6	do	64.0	300	400		90	75	400	6 grams phytin	4	2,600	41
7	do	64.0	300	400		90	75	300	12 grams egg-albumin	4	2,600	41
8	Medical student F	54.0	400		120	40		175	150 grams bananas 200 grams rice cake	4	2,100	39
9	Prisoner 4017 (D.)	43.5	200	150	75	150		500		6	1,650	38
10	Prisoner A	52.5	300	300	40	50		300		4	1,880	37
11	do	52.5	250	250	40	100		300	75 grams rice polishings	4	1,880	37
12	Prisoner 6917 (E.)	54.0	250	200	100	100		500		3	1,750	31
13	do	54.0	250	200	100	100		500		3	1,750	31
14	Prisoner 18736 (G.)	45.9	200	200	60	50		500		4	1,350	30
15	Prisoner 8110 (H.)	53.0	250	200	60	75		500		4	1,600	30

[a] Red rice, husked only.

Experiment number.	Subject.	Nitrogen—									Phosphoric acid.						
		Intake.				Outgo—				Balance of nitrogen.	Intake.		Outgo—				Balance of P_2O_5.
		Animal nitrogen.	Vegetable nitrogen.	Total.	Per kilogram of body weight.	In urine.		In fæces.			Total P_2O_5	Per kilogram of body weight.	In urine.		In fæces.		
						Nitrogen.	Per cent of intake.	Nitrogen.	Per cent of intake.				P_2O_5.	Per cent of intake.	P_2O_5.	Per cent of intake.	
		Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*		*Grams.*		*Grams.*	*Grams.*	*Grams.*	*Grams.*		*Grams.*		*Grams.*
1	Medical student V	0.49	5.49	5.98	0.11	5.87	98.2	1.77	29.1	−1.66	-------	-------	-------	-------	-------	-------	-------
2	Medical student F	0.49	5.60	6.09	0.11	4.86	90.1	2.06	33.3	−0.83	-------	-------	-------	-------	-------	-------	-------
3	Prisoner 20560 (C.)	0.38	5.45	5.83	0.12	4.26	73.1	3.11	-------	−1.68	3.22	0.066	1.03	32.0	2.03	63.1	+0.16[a]
4	do	0.38	4.53	4.91	0.10	4.26	86.7	2.33	-------	−1.50	1.42	0.029	1.04	73.2	0.83	58.4	−0.45[b]
5	Prisoner B	0.31	9.26	9.57	0.15	10.85	>100.0	3.39	35.4	−4.67	1.50	0.023	1.74	>100	0.73	48.7	−0.97
6	do	0.25	9.16	9.41	0.15	8.26	87.7	3.23	34.3	−2.08	4.91	0.077	1.56	31.7	1.85	37.7	+1.50
7	do	1.95	9.11	11.06	0.17	9.66	87.3	3.38	30.6	−1.98	1.88	0.029	1.57	83.5	0.61	32.4	−0.30
8	Medical student F	4.26	5.66	9 92	0.18	8.20	82.6	2.15	21.7	−0.43	-------	-------	-------	-------	-------	-------	-------
9	Prisoner 4017 (D.)	2.84	4.76	7.60	0.18	5.90	77.5	1.64	21.6	+0.06	1.28	0.033	0.80	62.5	0.49	38.0	−0.01
10	Prisoner A	2.52	8.47	10.99	0.21	7.97	72.5	2.64	24.0	+0.38	1.57	0.032	1.14	72.6	0.52	33.7	+0.01
11	do	2.52	8.97	11.49	0.23	7.18	62.5	3.74	-------	+0.57	5.46	0.104	1.38	25.3	3.46	63.4	+0.62
12	Prisoner 6917 (E.)	4.37	5.90	10.27	0.19	8.96	87.4	2.03	19.0	−0.72	1.77	0.033	1.23	57.6	0.76	42.9	−0.22
13	do	4.37	5.90	10.27	0.19	8.73	85.1	2.27	22.1	−0.73	1.77	0.033	1.11	57.6	0.73	41.2	−0.10
14	Prisoner 18736 (G.)	2.62	5.28	7.90	0.17	6.72	85.1	1.98	25.1	−0.80	1.45	0.032	0.95	65.5	0.58	40.0	−0.08
15	Prisoner 8110 (H.)	2.62	5.90	8.52	0.16	6.87	80.6	(c)	(c)	-------	1.63	0.031	1.21	74.2	(c)	(c)	-------

[a] Red rice, husked only. [b] White rice, polished. [c] Limitation of fæces a failure. Figures for urine only.

The detailed records of five of these experiments have already been published in a previous paper[7] (Nos. 5, 6, 7, 10, and 11), while a complete record of the remaining ten will be found at the end of this paper.

NITROGEN METABOLISM.

In their experiments with a rice diet, neither Rübner nor Thomas was able to establish nitrogen equilibrium in spite of a comparatively great intake of calories. However, they experimented only with Europeans. The nitrogen excretion likewise exceeded the nitrogen taken with the food in our experiments numbered 1 to 4, made on Malays. Kumagawa succeeded in establishing nitrogen equilibrium in only 1 experiment, in which 8 grams of nitrogen, or 0.19 gram per kilogram and 2,590 calories per kilogram were consumed. However, it must not be forgotten that in addition he ate considerable quantities of turnips and other foodstuffs. (See Table III.) Therefore, in metabolism experiments carried on by our method, it appears to be difficult to give the amount of nitrogen needed by the body by means of a diet of pure rice, with such additions as sugar, bacon, and bananas as are shown in Table IV. It might be easier to give the quantities of protein necessary to establish nitrogen equilibrium if the subjects were performing muscular labor with a resulting greater demand on energy than was the case with our volunteers who practically did no work. A number of experiments on metabolism in which mixed diets of rice and fish, or rice and meat and vegetables, were given, have been performed, but it would take too much space to review all of these.[8] Therefore, we will limit our discussion to experiments numbered 5 to 15 in our series. We employed in these instances varying quantities of nitrogen as well as varying numbers of calories.

To facilate comparison we have reduced the figures for calories, the nitrogen intake, and the nitrogen balance, to a uniform standard of 50 kilograms of body-weight and these values are given in Table V.

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[7] *Loc. cit.*

[8] W. O. Atwater and C. F. Longworthy, *Bull. U. S. Dept. Agr. Off. Exp. Sta.* (1898), **No. 45.** C. Cijkmann, *Virchow's Arch.* (1893), **131**, 147, 180. Taniguti, *Arbeit a. d. kais. jap. militär Med.* Lebraust, 1, 85, 90. *Review Malys Jahresbericht* (1892), **22**, 467-8.

TABLE V.—*The figures from Table IV reduced to 50 kilograms body weight.*

Number of experiment.	Calories.	Nitrogen intake.	Nitrogen balance.	Diet.
		Per cent.	*Per cent.*	
4	2,250	5.0	−1.6	Rice, bacon, sugar, coffee, bananas, rice cake.
2	2,200	5.5	−0.8	
1	2,200	5.5	−1.7	
3	2,200	6.0	−1.6	
5	2,050	7.5	−3.5	Rice, bread, bacon, sugar, coffee, phytin (No. 6), egg albumin (No. 7).
6	2,050	7.5	−1.6	
7	2,050	8.5	−1.5	
14	1,500	8.5	−0.9	Rice, bread, fish, sugar, coffee, and bananas, rice cake (No. 8), rice polishings (No. 11).
8	1,950	9.0	−0.4	
9	1,900	9.0	+0.1	
12	1,550	9.5	−0.6	
13	1,550	9.5	−0.6	
10	1,850	10.5	+0.4	
11	1,850	11.5	+0.5	

It was not possible to establish nitrogen equilibrium, even in cases where the nitrogen intake was comparatively high, if the number of calories taken was below 1,800 for 50 kilograms of body weight (see experiments numbered 12, 13, 14); on the other hand, if the number 1,800 was equalled or exceeded, then 9 grams of nitrogen per 50 kilograms of body weight were sufficient. If a less quantity of nitrogen than the above figure was taken with the food, then the loss of nitrogen exceeded the amount taken, even if the number of calories reached 2,200. However, with an intake of 5 to 6 grams of nitrogen, the deficit amounted to less than 2 grams (see experiments numbered 1 to 4). Consequently, we are justified in concluding that in some instances 8 grams of nitrogen per 50 kilograms, or 0.16 gram per kilogram of body weight are sufficient This value is higher than the lowest limit found by other authors, who succeeded in establishing nitrogen equilibrium on 0.1 gram per kilogram of body weight.

The question as to whether the quantity of protein given in a certain food will be sufficient or not, is mainly influenced by the proportion of protein absorbed, for that protein which is excreted, unabsorbed, by means of the fæces, obviously is worthless for the nutrition of the body. If from this point of view we consider the nitrogen absorption in our experiments, it appears that a subject, given a diet of pure rice, excretes in the fæces about 30 per cent (experiments numbered 1 and 2), and even

47 per cent (experiment number 4) of the nitrogen taken. The nitrogen contained in the outer layers of the rice, whether taken with the grain (experiment number 3), or in the form of the material milled off from the grain and added to the food (experiment number 11) is absorbed only to the extent of about 25 per cent. Therefore, the fact that the nitrogen content of red rice is somewhat higher than that of the white variety is of no physiological importance.

Rübner found about 75 per cent of the nitrogen in rice to be absorbed, Mori as much as 78.3 per cent. On the other hand, Thomas and Yukawa give values agreeing more closely with ours, namely, 67.4 per cent and 66.1 per cent, respectively.

The nitrogen in the fæces does not consist solely of that not absorbed from the foodstuffs, but it is partly present as nitrogen from the secretions of the intestinal tract. Therefore, the quantity of unabsorbed nitrogen, expressed in percentage of the nitrogen taken, must be proportionally higher the lower the nitrogen intake is. This explains the fact that not only in our work, but also in that of Thomas[9] and Yukawa,[10] a lower percentage of the nitrogen taken was found to be absorbed, even with a lower nitrogen intake, than was the case in the experiments of Rübner,[11] Kellner, and Mori. The percentage of absorption of nitrogen when the diet consists of bread and rice is practically the same as in the case of white rice alone. This is made plain by comparing experiments numbered 5 to 7 with numbers 1 and 2.

The experiments with mixed diet are interesting, as is shown by Table VI.

TABLE VI.—*Experiments with mixed diet.*

Experiment number.	In the food.			Quantity of nitrogen in fæces.	Absorption of nitrogen.	Nitrogen of fæces in per cent of animal nitrogen in food.
	Animal nitrogen.	Vegetable nitrogen.	Total nitrogen.			
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Per cent.*	*Per cent.*
5	0.31	9.26	9.57	3.39	64.6	36.5
6	0.25	9.16	9.41	3.23	65.7	35.2
7	1.95	9.11	11.06	3.38	69.4	37.1
10	2.52	8.47	10.99	2.64	76.0	31.2
13	4.37	5.90	10.27	2.27	77.9	38.5
12	4.37	5.90	10.27	2.03	81.0	36.0
8	4.26	5.66	9.92	2.15	78.3	37.9
14	2.62	5.28	7.90	1.98	74.9	37.5
9	2.84	4.76	7.60	1.64	78.4	34.4

The smaller the proportion of vegetable nitrogen in the food the greater relatively, is the absorption of nitrogen. The nitrogen content of the fæces is practically directly proportional to the quantity of vegetable nitrogen in the food, and is influenced but little by the amount in the animal constituents. For this reason, the quantity of nitrogen in the fæces, expressed in percentages of the vegetable nitrogen in the food, is practically a constant value of 35 per cent. Therefore, it is necessary to know the relative value between the protein of animal and of vegetable origin in order to determine the physiologic value of the protein content of a given food. Nearly one-third of the protein from rice and wheat passes unabsorbed through the intestines into the fæces; this must be subtracted as physiologically valueless.

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[9] *Loc. cit.*

[10] *Arch. Verd. Krcht.* (1910) **15**, 4–5.

[11] *Ztschr. Biol.* (1889) **25**, 102–22.

According to the recent investigations by Michaud[12] and by Thomas,[13] the biologic value of various classes of proteins differs widely. Therefore, protein in rice certainly would have a lower biologic value than protein from animal sources. It is to be regretted that our knowledge of the chemical composition of the protein of rice is so meager, whereas many detailed investigations on other vegetable proteins of considerably minor importance exist.

Finally, we wish to emphasize the fact that the absorption of nitrogen can be determined only by an exact knowledge of the nitrogen content of the fæces. The quantity of the nitrogen absorbed is equal to the quantity ingested, minus the amount in the fæces. The quantity of nitrogen excreted in the urine is equal to the amount absorbed only in case the subject is in exact nitrogenous equilibrium, so that miscalculations would result in many instances if the former figures were to be taken.

PHOSPHORUS METABOLISM.

A summary of our experiments is given in Table VII; the quantity of phosphorus ingested and the phosphorus balance being reduced to 50 kilograms of body weight.

TABLE VII.—*Phosphorus metabolism reduced to 50 kilograms body weight.*

Experiment number.	P_2O_5 intake.	P_2O_5 balance.
	Per cent.	*Per cent.*
5	1.15	—0.8
4	1.45	—0.45
7	1.45	—0.3
10	1.60	+0.01
14	1.60	—0.10
9	1.65	—0.01
12	1.65	—0.2
13	1.65	—0.1
3	3.80	+0.16
6	3.85	+1.2
11	5.20	+0.6

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[12] *Ztschr. f. phys. Chem.* (1907), **59**, 405–91.

[13] *Loc. cit.*

While it is not possible to establish an exact phosphorus minimum, just as it is impossible to establish a nitrogen minimum, our experiments show that an intake of less than 1.65 grams of phosphorus per 50 kilograms of body weight, or 0.033 grams phosphoric anhydride per kilogram is insufficient to cover the demand of the body for phosphorus. The quantities given by most authors [14] are considerably higher than this figure.

We discussed the fact that the phosphorus content of rice is of very great physiologic importance, in the introduction to this paper. Experiments numbered 4, 5, and 7 show conclusively that a diet consisting of white rice, bread, bacon, and other foodstuffs poor in phosphorus does not cover the demand of the body for that element, even with the addition of small quantities of fish. (See experiments numbered 12, 13, and 14.) However, the phosphorus balance becomes positive if unpolished rice, rice bran, or phytin is added to the food. (See experiments numbered 3, 6, and 11.) A comparison of experiments numbered 3 and 4 very plainly shows that the body loses phosphorus when on a diet consisting mainly of white rice, while on the same diet, red rice being substituted, the amount of that element exceeds the demand of the body. The distribution of phosphorus in urine and fæces is not of as great importance as in the case of nitrogen, because the phosphorus excreted in the fæces consists in part of that which has previously been absorbed and again excreted in the lower parts of the large intestines. It should be noted that those experiments where the amount of phosphorus taken was high show that the excess is excreted almost entirely in the fæces.

Our figures seem to show that the phosphorus demand of Filipinos (Malays) is lower than that given by other authors. It may be that the body becomes accustomed to a lower supply of phosphorus than that usually considered to be necessary, and the high results in the metabolism experiments on Europeans might simply be requirements of "luxury." Another view seems not too far removed: Several authors have argued that there is a connection between mental work and phosphorus metabolism. Of course, the belief of older investigators, that mental work increases metabolism in the brain, which is rich in phosphorus, has been shown to be absurd. The quantity of phosphorus in the brain itself is much too small as a whole to influence the phosphorus metabolism of the body. However, the nervous system might have a regulatory influence on all the dissimilative processes in the body, so that what we might term the "tone" of the "catabolic cell-metabolism" could be increased during intensive activity of the nervous system and lowered during rest. We have based this consideration especially on the very striking results of experiments by Folin and Shaffer. If such a view has any basis in fact, then the "tone" of the cell metabolism of the ordinary Malay, with his phlegmatic temperament, must be very low. Such a conclusion is somewhat startling. Once considered, it was necessary to attempt to demonstrate whether a difference in the destruction of the body material containing phosphorus takes place in ordinary Filipinos, as shown by an increased excretion of phosphorus in the urine, during periods of mental work and control periods, in which the subjects are left entirely to themselves, the diet being the same. With three prisoners (D, E, H) we divided the entire period into three sub-periods, during which on the one hand the men were either quiet, by themselves or, on the other hand, performed mental work. It was very easy to obtain "mental rest," but much more difficult to secure "mental work." The men were asked to study arithmetic or history, or to read poems either in Tagalog, English, or Spanish, but it was almost impossible to make them do this for more than two or three hours a day, and we are sure that when we were not present there was very little work done. One experiment, number 9, apparently gave a decidedly positive result; experiment number 15 was practically indecisive; and experiments numbered 12 and 13, which seemed to be the most successful of all, apparently gave exactly the opposite result, if differences could be considered to be present at all.

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[14] Oeri, *Ztschr. klin. Med.* (1909), **67**, 288, 306; Ehvstrohm, *Skand. Arch. Phys.* (1903), **14**, 91; Renoall, *Skand. Arch. Phys.* (1905), **16**, 94-138; R. Tigerstedt, *Handbuch d. Phys.* (Nagel) (1908), **1**, 530.

Table VIII shows the influence of mental work and mental rest on the phosphorus excretion in the urine, so far as our experiments go. We do not believe that any definite conclusion can be drawn from them.

TABLE VIII.—*Nitrogen and phosphoric anhydride excretion in the urine during periods of rest and mental work.*

Experiment 9, Prisoner D.

Condition.	Nitrogen.	P_2O_5.
	Grams.	*Grams.*
Rest 2 days	11.01	1.42
Mental work 2 days	12.96	1.85
Rest 2 days	11.41	1.56

Experiment 15, Prisoner H.

Condition.	Nitrogen.	P_2O_5.
	Grams.	*Grams.*
Rest 2 days	13.75	2.14
Mental work 2 days	13.77	2.42

Experiments 12–13, Prisoner E.

Condition.	Nitrogen.	P_2O_5.
Rest 3 days:	*Grams.*	*Grams.*
First day	9.09	1.65
Second day	8.80	1.13
Third day	9.01	0.91
Mental work 3 days:		
First day	8.66	1.08
Second day	8.41	0.98
Third day	9.13	1.28
Rest 2 days:		
First day	8.45	1.29
Second day	8.76	1.25

TABLE IX.—*Complete records of the experiments and analyses of foodstuffs.*

Numbers of experiments.	Foodstuff.	N.	P_2O_5.
		Per cent.	*Per cent.*
9, 12, 13, 14, 15	Bread	1.28	0.17
1, 2	Rice I	1.109	
3	Rice r	1.29	0.77
4	Rice w	1.26	0.52
8	Rice II	1.116	
9	Rice A	1.29	0.28
12, 13, 14, 15	Rice B	1.23	0.37
1, 2	Rice cake I	0.405	(a)
8	Rice cake II	0.423	
1, 2	Bacon I	0.82	(b)
3, 4	Bacon II	0.75	0.056
8	Fish (dalag)	3.556	
9	Fish (salmon)	3.60	0.40
12, 13, 14, 15	Salt codfish	4.87	0.346
3, 4	Onions	0.14	0.06
		Gram.	*Gram.*
1, 2, 8	Coffee 150 cc	0.02	
3, 4, 9, 12, 13, 14, 15	Coffee 500 cc	0.02	0.016

[a] Fat 12.2 per cent.

[b] Fat 86 per cent.

EXPERIMENT No. 1.—*September 22 to 24, 1910 (subject, medical student V; weight, 53.7 kilograms).*

Daily food.	Nitrogen.	Calories (estimated).
	Grams.	
400 grams rice I	4.44	1,300
200 grams rice cake I	0.81	340
100 grams bananas	0.22	80
45 grams sugar		160
60 grams bacon I	0.49	480
150 grams coffe	0.02	
Total intake per day	5.98	2,360

Day.	Analyses of urine.		Analyses of fæces.
	Quantity.	Nitrogen.	
	cc.	*Per cent.*	
September 22	530 (1,000)	6.09	61.5 grams fæces containing 8.80 per cent nitrogen and 94.9 per cent solids. Total nitrogen, 5.31 grams.
September 23	560 (1,000)	5.75	
September 24	880 (1,000)	5.76	
Total nitrogen		17.60	

Balance sheet, per day.

	Grams of nitrogen.
Outgo in urine	5.87
Outgo in fæces	1.77
Total outgo	7.64
Intake, total	5.98
Balance	1.66

EXPERIMENT No. 2.—*September 22 to 24, 1910 (subject, medical student F; weight, 54.0 kilograms).*

Daily food.	Nitrogen.	Calories (estimated).
	Grams.	
400 grams rice I	4.44	1,300
200 grams rice cake I	0.81	340
150 grams bananas	0.33	120
45 grams sugar		160
60 grams bacon I	0.49	480
150 grams coffee	0.02	
Total intake per day	6.09	2,400

Day.	Analyses of urine.		Analyses of fæces.
	Quantity.	Nitrogen.	
	cc.	*Per cent.*	
September 22	930	4.87	75.0 grams dried fæces containing 8.28 per cent nitrogen and 94.6 pet cent solids. Total nitrogen, 6.19 grams.
September 23	1,075	4.92	
September 24	725	4.78	
Total nitrogen		14.57	

Balance sheet, per day.

	Grams of nitrogen.
Outgo in urine	4.86
Outgo in fæces	2.06
Total outgo	6.92
Intake, total	6.09
Balance	−0.83

EXPERIMENT NO. 3.—*November 16 to 21, 1910 (subject, prisoner C; weight, 49.0 kilograms).*

Daily food.	Nitrogen.	P_2O_5.	Calories (estimated).
	Grams.	*Grams.*	
400 grams rice r	5.16	3.08	1,350
100 grams onions	0.14	0.06	50
50 grams bacon H	0.38	0.03	350
300 grams coffee	0.15	0.05	
20 grams lard			150
50 grams sugar			200
Total intake per day	5.83	3.22	2,200

Day.	Analyses of urine.			Analyses of fæces.
	Quantity.	Nitrogen.	P_2O_5.	
	cc.	*Per cent.*	*Per cent.*	
November 18	640	17.05	4.129	204 grams dried fæces containing 6.11 per cent nitrogen and 3.97 per cent P_2O_5. Total, 12.46 grams nitrogen and 8.10 grams P_2O_5.
November 19	400			
November 20	400			
November 21	480			

Balance sheet, per day.

	Grams of nitrogen.	Grams of P_2O_5.
Excretion in urine	4.26	1.03
Outgo in fæces	3.11	2.03
Total outgo	7.37	3.06
Intake, total	5.83	3.22
Balance	−1.54	+0.16

EXPERIMENT NO. 4.—*November 22 to 25, 1910 (subject, prisoner C; weight, 48.6 kilograms).*

Daily food.	Nitrogen.	P_2O_5.	Calories (estimated).
	Grams.	*Grams.*	
400 grams rice W	4.24	1.28	1,350
100 grams onions	0.14	0.06	
50 grams bacon H	0.38	0.03	350
300 grams coffee	0.15	0.05	
20 grams lard			150
50 grams sugar			200
Total intake per day	4.91	1.42	2,200

Day.	Analyses of urine.			Analyses of fæces.
	Quantity.	Nitrogen.	P_2O_5.	
	cc.	*Grams.*	*Grams.*	
November 22	2,425	17.04	4.16	132.4 grams dried fæces containing 7.05 per cent nitrogen and 2.50 per cent P_2O_5. Total, 9.33 grams nitrogen and 3.31 grams P_2O_5.
November 23	1,305			
November 24	850			
November 25	980			

Balance sheet, per day.

	Grams of nitrogen.	Grams of P_2O_5.
Outgo in urine	4.26	1.04
Outgo in fæces	2.33	0.83
Total outgo	6.59	1.87
Intake, total	4.91	1.42
Balance	−1.68	−0.45

EXPERIMENT No. 8.—*September 27 to October 1, 1910 (subject, medical student F; weight, 54.0 kilograms).*

Daily food.	Nitrogen.	Calories (estimated).
	Grams.	
400 grams rice II	4.46	1,300
120 grams fish (dalag)	4.26	100
150 grams bananas	0.33	120
200 grams rice cake II	0.85	340
40 grams sugar		160
150 grams coffee	0.02	
10 grams lard		80
Total intake per day	9.92	2,100

Day.	Analyses of urine.		Analyses of fæces.
	Quantity.	Nitrogen.	
	cc.	*Grams.*	
September 27	690	7.37	46 grams dried fæces containing 6.85 per cent nitrogen and 98 grams dried fæces containing 7.73 per cent nitrogen. Total, 10.73 grams nitrogen (5 days).
September 28	915	7.60	
September 29	1,475	9.09	
September 30	1,315	8.71	
October 1	Lost.		
Total		[a] 32.77	

[a] 4 days.

Balance sheet, per day.

	Grams of nitrogen.
Outgo in urine	8.20
Outgo in fæces	2.15
Total outgo	10.35
Intake, total	9.92
Balance	−0.43

EXPERIMENT No. 9.—*June 27 to July 3, 1910 (subject, prisoner D; weight, 43.5 kilograms).*

Daily food.	Nitrogen.	P_2O_5.	Calories (estimated).
	Grams.	*Grams.*	
200 grams rice A	2.57	0.56	650
150 grams bread	1.93	0.24	300
75 grams salmon	2.84	0.32	100
150 grams sugar	----------	----------	600
500 grams coffee	0.26	0.16	----------
Total intake per day	7.60	1.28	1,650

Day.	Analyses of urine.			Analyses of fæces.
	Quantity.	Nitrogen.	P_2O_5.	
	cc.	*Grams.*	*Grams.*	
June 27	1,110	11.01	1.419	140 grams dried fæces containing 7.02 per cent nitrogen and 2.08 per cent P_2O_5. Total, 9.83 grams nitrogen and 2.91 grams P_2O_5.
June 28	980			
June 29	890	12.96	1.848	
June 30	855			
July 1	1,145	11.41	1.563	
July 2	680			
Total	----------	35.38	4.830	

Balance sheet, per day.

	Grams of nitrogen.	Grams of P_2O_5.
Outgo in urine	5.90	0.80
Outgo in fæces	1.64	0.49
Total outgo	7.54	1.29
Intake, total	7.60	1.28
Balance	+0.06	−0.01

EXPERIMENT No. 12.—*July 23 to 25, 1910 (subject, prisoner E; weight, 54.0 kilograms).*

Daily food.	Nitrogen.	P_2O_5.	Calories (estimated).
	Grams.	*Grams.*	
250 grams rice B	3.075	0.925	800
200 grams bread	2.56	0.34	400
100 grams salt codfish	4.37	0.346	150
100 grams sugar	----------	----------	400
500 grams coffee	0.26	0.16	----------
Total intake per day	10.265	1.77	1,750

EXPERIMENT No. 12.—*July 23 to 25, 1910 (subject, prisoner E; weight, 54.0 kilograms)*—Continued.

Day.	Analyses of urine.			Analyses of fæces.
	Quantity.	Nitrogen.	P_2O_5.	
	cc.	*Grams.*	*Grams.*	
July 23	2,030	9.09	1.65	74 grams containing 8.26 per cent nitrogen and 3.01 per cent P_2O_5. Total, 6.112 grams nitrogen and 2.29 grams P_2O_5.
July 24	1,250	8.80	1.13	
July 25	830 (1,000)	9.01	0.91	
Total		26.90	3.69	

Balance sheet, per day.

	Grams of nitrogen.	Grams of P_2O_5.
Outgo in urine	8.96	1.23
Outgo in fæces	2.03	0.76
Total outgo	10.99	1.99
Intake, total	10.27	1.77
Balance	−0.72	−0.22

EXPERIMENT No. 13.—*July 26 to 30, 1910 (subject, prisoner E; weight, 54 kilograms).*

Daily food.	Nitrogen.	P_2O_5.	Calories (estimated).
	Grams.	*Grams.*	
250 grams rice B	3.075	0.925	800
200 grams bread	2.56	0.34	400
100 grams salt codfish	4.37	0.346	150
100 grams sugar			400
500 grams coffee	0.26	0.16	
Total intake per day	10.265	1.77	1,750

Day.	Analyses of urine.			Analyses of fæces.
	Quantity.	Nitrogen.	P_2O_5.	
	cc.	*Grams.*	*Grams.*	
July 26	660 (1,000)	8.66	1.08	95 grams dried fæces containing 7.18 per cent nitrogen and 2.30 per cent P_2O_5. Total, 6.82 grams nitrogen and 2.19 grams P_2O_5.
July 27	640 (1,000)	8.41	0.98	
July 28	670 (1,000)	9.13	1.28	
July 29	600 (1,000)	8.45	1.29	
July 30	580 (1,000)	8.76	1.25	

Balance sheet, per day.

	Grams of nitrogen.	Grams of P_2O_5.
Outgo in urine	8.73	1.11
Outgo in fæces	2.27	0.76
Total outgo	11.00	1.87
Total intake	10.27	1.77
Balance	−0.73	−0.10

Experiment No. 14.—*August 10 to 14, 1910 (subject, prisoner G; weight, 45.9 kilograms).*

Daily food.	Nitrogen.	P_2O_5.	Calories (estimated).
	Grams.	*Grams.*	
200 grams rice B	2.46	0.74	650
200 grams bread	2.56	0.34	400
50 grams sugar	----------	----------	200
60 grams salt codfish	2.62	0.21	100
500 grams coffee	0.26	0.16	----------
Total intake per day	7.90	1.45	1,350

Day.	Analyses of urine.			Analyses of fæces.
	Quantity.	Nitrogen.	P_2O_5.	
	cc.	*Grams.*	*Grams.*	
August 11	830	26.88	3.796	114 grams dried fæces containing 6.93 per cent nitrogen and 2.04 per cent phosphoric anhydride. Total, 2.33 grams phosphoric anhydride and 7.90 grams nitrogen.
August 12	1,020			
August 13	1,570			
August 14	1,550			

Balance sheet, duration 4 days.

	Grams of nitrogen.	Grams of phosphoric anhydride.
Outgo urine	6.72	0.949
Outgo fæces	1.985	0.583
Total outgo	8.705	1.532
Total intake	7.90	1.45
Balance	−0.80	−0.08

EXPERIMENT No. 15.—*June 16 to 21, 1910 (subject, prisoner H; weight, 53.0 kilograms).*

Daily food.	Nitrogen.	P_2O_5.	Calories. (estimated).
	Grams.	*Grams.*	
250 grams rice B	3.08	0.93	800
200 grams bread	2.56	0.34	400
60 grams salt codfish	2.62	0.21	100
75 grams sugar			300
500 grams coffee	0.26	0.16	
Total intake per day	8.52	1.64	1,600

Analyses of urines.

Day.	Quantity.	Nitrogen.	Phosphoric anhydride.
	cc.	*Grams.*	*Grams.*
June 17	1,050	7.30	
June 18	840	13.75	2.135
June 19	725		
June 20	550	13.77	2.42
June 21	1,030		

Balance sheet, duration 4 days.

	Grams of nitrogen.	Grams of phosphoric anhydride.
Outgo urine	6.87	1.21
Outgo fæces		
Total outgo		
Total intake	8.52	1.63
Balance		

THE EFFECT OF ULTRA-VIOLET RAYS ON AMŒBÆ, AND THE USE OF THESE RADIATIONS IN THE STERILIZATION OF WATER.[1]

By WESTON P. CHAMBERLAIN and EDWARD B. VEDDER.[2]

(From the United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands.[3])

It has already been shown by several investigators that the ultra-violet rays of the spectrum are capable of killing bacteria in a few seconds, and the Westinghouse Cooper Hewitt Company of London and Paris have constructed several types of practical water sterilizers utilizing these rays. The ultra-violet radiation, upon which the action of these sterilizers depends, is produced by a quartz mercury-vapor lamp either suspended over or immersed in the water. One type has a capacity of 132,000 gallons (528,000 liters) of potable water in twenty-four hours, and only requires an electric current of three or three and one-half amperes at 220 volts for its operation. It has been demonstrated by experimentally polluting water with *Bacillus coli* that these organisms are killed during the passage through such a sterilizer, although the water remains in the apparatus for only five seconds. A more complete description, together with photographs and diagrams of this apparatus, may be obtained from the articles of Foulds(1) and Thresh and Beale.(2)

There are many features about this method of sterilization that render it peculiarly suitable for use in tropical countries and by armies in the field, among which the following may be mentioned. It is automatic and can be intrusted to a comparatively unskilled man, this automatic action being secured by a valve in the inlet which is operated by the current of the same circuit that produces the rays, and which prevents any water from entering the sterilizer if the current is interrupted. The apparatus is small and one type is portable. The water is neither heated nor altered in taste by the process. It provides a rapid method by which a badly polluted water may be transformed into one of potable quality, and finally it is very economical in operation. The low cost should place it within the means even of poor native villages, a small gasoline engine being capable of producing sufficient power.

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[1] Published by permission of the Chief Surgeon, Philippines Division.

[2] W. P. Chamberlain, major, Medical Corps, United States Army, and E. B. Vedder, captain, Medical Corps, United States Army, members of the United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands.

[3] The apparatus for these experiments was supplied by the Bureau of Science, and the exposures were made in that Bureau.

For army use this apparatus with dynamo and gasoline engine could be readily carried in an escort wagon or automobile. In case the latter were used a special gasoline engine could be dispensed with, and the motor of the automobile utilized to drive the dynamo. The small type of apparatus delivers 130 gallons (520 liters) of sterile water per hour, and it would be capable of supplying an entire regiment with a quart of water for each man after only three hours' operation. The larger apparatus, although designed for permanent installation, could also be carried in an escort wagon, and would supply 132,000 gallons (528,000 liters) in twenty-four hours, thus providing in twelve hours a little over 3 gallons (12 liters) of sterile water for every soldier in a division of 20,000 men. The delivery of pure water would commence within a few minutes after arrival in camp, would be continuous, and the water would be unchanged in taste, a great desideratum when dealing with soldiers whose objection to the flat taste of boiled water is well known. Also there would be no time wasted in waiting for water to cool as is the case when sterilization by heat is resorted to.

But in considering the use of such a sterilizer in the Tropics we are confronted by the fact that bacterial pollution is not the only nor, in some cases, the most important danger that lurks in the drinking supply. Amœbæ are found in practically all tropical surface waters, and while the free-living species are not pathogenic, it is to be expected that *Entamœba histolytica* will be encountered under the same conditions of pollution that would cause the presence of typhoid bacilli or cholera spirilla in the water. In similar circumstances, *Balantidium coli*, *Lamblia intestinalis*, and other protozoa, as well as the ova or larvæ of various parasites are liable to occur. Therefore it is important, before adopting any method of water purification for tropical countries, to show that the procedure will protect the consumers against infections with animal parasites as well as against the bacterial causes of disease. Filter beds will eliminate 99 per cent of the bacteria in water, but will not remove amœbæ or other protozoa, so that this method of water purification is impracticable in the Philippines for this reason and also because of its great expense.

It seems quite probable, from what we know of the action of the ultra-violet radiation, that protozoa and the ova or larvæ of intestinal worms may all be killed by this powerful agent. It has been shown(3) that radium emanations on short exposures inhibit, and on longer exposures kill various forms of bacteria, protozoa, ova, embryos, and larvæ. The only reference we have found indicating that the effects of ultra-violet rays have been tested with protozoa is an article by Hertel(4) showing that paramœcia are killed by these short length waves. We have failed to find in Manila any reference indicating that the influence of ultra-violet rays on amœbæ has been investigated.

These considerations seemed to indicate the desirability of studying the effect of ultra-violet rays on amœbæ and the experiments about to be described were undertaken for this purpose. The source of the ultra-violet radiations used by us was a quartz mercury-vapor arc lamp owned by the Bureau of Science in Manila. This lamp, which was made by the firm of W. C. Heraeus, Hanau, Germany, was rather small for our purpose, but produced a brilliant light particularly rich in ultra-violet rays. A description of the lamp may be found in the Zeitschrift für Electro-chemie.(5)

The strain of amœbæ used was originally isolated from a normal human stool, has been kept constantly under cultivation for a year, and is the same that was employed previously to determine the efficacy of the ipecac treatment of dysentery.(6) These amœbæ belong undoubtedly to a free-living species, but it is believed that any agent which is found to be detrimental to the growth of such free-living organisms will prove even more efficacious against the parasitic amœbæ which are much more susceptible to unfavorable conditions as shown by the fact that it has hitherto been impossible to cultivate them on artificial media.

The experiments were performed in a number of ways, using both solid and fluid media, and with exposures for different periods of time at various distances from the source of radiation. After the exposure, cultures were made at once from the exposed amœbæ to determine whether they had been killed by the rays, and these observations were controlled by similar cultures made from amœbæ grown under identical circumstances, but which had not been subjected to the action of ultra-violet waves. The effect of the rays on the amœbæ has also been compared with the effect produced on *Bacillus typhosus* and *Bacillus dysenteriæ* (Shiga) by similar exposures. Before proceeding to an account of the experiments themselves we will give a brief description of the stock culture of amœbæ and the plates used, and the method of exposing the cultures to the rays.

A. *Stock culture of amœbæ.*—The amœbæ referred to previously were cultivated for several weeks in a 250 cubic centimeter Erlenmeyer flask containing plain bouillon mixed with double the quantity of distilled water. During this period the flask was handled with care so that the fluid was never shaken, and the amœbæ that developed were found in countless numbers lying in the surface film of the bouillon. This flask was then used as a stock culture from which all the inoculations of an experiment were made, and a single large loopful taken from this surface film was used for each inoculation.

B. *Plates.*—The lamp already referred to is in the form of a small arc and the rays, both visible and invisible, radiate from this arc in every direction. The radiations diminish in intensity as the square of the distance from the source, and therefore it was desirable to make the exposures quite close to the lamp, approximately 10 centimeters from it. But it seemed probable, if ordinary-sized Petri dishes were used at this distance, that the outer margins of the plate might be beyond the sphere of effective intensity of the rays, and that for this reason some of the amœbæ might not be killed. In order to avoid this difficulty we used special plates consisting of aluminum ointment boxes 2.5 centimeters in diameter and with tightly fitting covers. These boxes were sterilized in the autoclave, filled with plain sterile agar and covered with their lids. This kind of plate avoided the difficulty just mentioned, and was very convenient to handle, took up less space in the incubator, and required much less agar, points by no means beneath consideration when it is a question of making several hundred exposures.

C. *Method of making exposures.*—The inoculated plate was uncovered and placed directly under the center of the lamp. The cover was turned upside down and placed beside the plate in order that it should also be exposed to the rays with a view to killing any amœbæ that might have contaminated it accidentally. The switch controlling the current operating the lamp was then closed and the appearance of the light timed with a watch. After the desired number of seconds of exposure, the switch was opened. We were thus enabled to time our exposures accurately. As soon the exposure was completed the cover was replaced on the plate.

This method was quite satisfactory except for one particular. We know that it is necessary for the lamp to become warmed up before it is capable of producing its maximum output of ultra-violet rays. This occurs a few seconds after the current is turned on, in which time the lamp becomes quite hot. It is probable that this temporary lack of efficiency in the apparatus when the current is first turned on explains why some of our cultures were not killed by exposure for a period of five seconds. This will be referred to again later.

In order to prove that the amœbæ and bacteria were killed by the ultra-violet rays, and not by heat generated by the lamp, we exposed a thermometer 10 centimeters from the lamp, that being the nearest point at which the cultures were ever placed. After a forty-second exposure the thermometer had risen to 50° centigrade. Thus it is shown that although the lamp itself may become very hot, sufficient heat to injure bacteria or amœbæ is not radiated to a distance of 10 centimeters in a period of forty seconds. Only a slight sensation of warmth is felt if the hand is exposed close to the lamp, although a severe burn might be caused if the lamp itself were actually touched.

Experiment I.—In this experiment plates made as described above were exposed to the ultra-violet rays immediately after they were inoculated. Part of these plates were inoculated with a loopful from the stock culture of amœbæ, part from a stock culture of *Bacillus typhosus,* and part from a stock culture of *Bacillus dysenteriæ.* A few of the plates inoculated with each organism were at once placed in the incubator to serve as controls while the remainder were exposed to the rays as described above, for periods varying from five to eighty seconds, and after the exposure they also were placed in the incubator. In order to compare the effects of the rays at different distances from their source, exposures were made at point 10, 15, and 23 centimeters from the lamp. After incubation for twenty-four hours, the plates were examined for growth and the results of this experiment are contained in Table I. In this as in subsequent tables, the plus sign means that growth occurred, while the minus sign indicates a sterile plate or culture.

TABLE I.—*Experiment 1: Agar plates exposed to ultra-violet rays immediately after inoculation, and then incubated 24 hours.*

Distance from lamp.	No.	Organism exposed.	Time of exposure in seconds.									Controls, not exposed.
			5	10	20	30	40	50	60	70	80	
A. 10 cm.	1	Amœbæ	—	—	—	—	—	—	—	—	—	+
	2	Amœbæ	—	—	—	—	—	—	—	—	—	+
	3	B. typhosus	—	—	—	—	—	—	—	—	—	+
	4	B. typhosus	+(6)	—	—	—	—	—	—	—	—	+
	5	B. dysenteriæ	+(2)	—	—	—	—	—	—	—	—	+
	6	B. dysenteriæ	—	—	—	—	—	—	—	—	—	+
B. 15 cm.	7	Amœbæ	—	—	—	—	—	—	—	—	—	+
	8	Amœbæ	—	—	—	—	—	—	—	—	—	+
	9	B. typhosus	—	—	—	—	—	—	—	—	—	+
	10	B. typhosus	—	—	—	—	—	—	—	—	—	+
	11	B. Dysenteriæ	+(1)	—	—	—	—	—	—	—	—	+
	12	B. Dysenteriæ	+(2)	—	—	—	—	—	—	—	—	+
C. 23 cm.	13	Amœbæ	+	+	+	+	+	+	+	—	—	+
	14	Amœbæ	+	+	+	+	+	+	+	—	—	+
	15	B. typhosus	+(3)	+	+	—	—	—	—	—	—	+
	16	B. typhosus	+(2)	+	+	—	—	—	—	—	—	+
	17	B. dysenteriæ	+(4)	+	+	—	—	—	—	—	—	+
	18	B. dysenteriæ	+(1)	+	+	—	—	—	—	—	—	+

The figures in parentheses in the 5-second column indicate the number of colonies found on the plate after incubation.

As is shown clearly in Table I, a profuse growth was found on all of the controls which were not exposed to the rays. Of the plates exposed for five seconds at 10 and 15 centimeters, some of those inoculated with bacteria showed growth and others were sterile, but none of the plates inoculated with amœbæ showed growth after this exposure. However, it will be seen that the effect of the rays on the bacteria was very pronounced even in five-second exposures, because instead of a profuse growth such as was observed on the control plates, only a few scattered colonies were found. If the lamp had been in preliminary operation for a few seconds, so that it was producing its maximum of rays at the time the exposure began, it is probable that all of the plates exposed for five seconds would have been sterile. It would have been very difficult however to make accurately timed exposures with the lamp continuously in operation, and in any case the results are sufficient for our present purpose. Others have already demonstrated that bacteria are killed in five seconds by such exposures. This experiment shows that in exposures close to the lamp amœbæ are destroyed by the ultra-violet radiations quite as readily as *Bacillus typhosus* or *Bacillus dysenteriæ.*

At a distance of 23 centimeters from the lamp it required twice as long an exposure to kill amœbæ as compared with *Bacillus typhosus* and *Bacillus dysenteriæ.* This is unimportant with regard to practical sterilization of water in the apparatus as manufactured, for the reason that in these sterilizers all the water is forced to flow close to the lamp, and furthermore the lamp used is probably much more powerful than the arc employed by us.

The conclusions which can clearly be drawn from this experiment are that amœbæ are destroyed by exposure for a few seconds to ultra-violet rays, and that at 10 centimeters distances they are as readily killed by this agent as are dysentery and typhoid bacilli.

However, it was desirable to determine whether encysted amœbæ would be killed by this method, since it is quite probable that amœbæ in a water supply would usually be present in the encysted form. The following experiment was performed for this purpose.

Experiment 2.—Plates were inoculated exactly as in the first experiment, but were incubated for twenty-four hours before exposure. During this period of growth many of the amœbæ became encysted. Since it is impossible to tell by microscopic examination whether an encysted amœba is dead or alive, this point was determined by cultures. After exposure to the ultra-violet rays the growth was scraped from the surface of the plate and inoculated into a tube containing a mixture of 1 part of plain bouillon and 2 parts water. This culture was incubated for twenty-four hours and then examined for motile amœbæ. Cultures were also made from control plates that had not been exposed to the light. The results of this experiment are shown in Table II.

TABLE II.—*Experiment 2: Agar plates inoculated with amœbæ and cultivated for 24 hours before exposure to ultra-violet rays. Plates contained encysted forms.*

Distance from lamp.	No.	Time of exposure in seconds.					Control, not exposed.
		10	20	30	40	50	
A. 10 cm.	1	—	—	—	—	—	+
	2	+	—	—	—	—	+
	3	—	—	+	—	—	+
	4	—	—	—	—	—	+
B. 15 cm.	5	—	—	—	—	—	+
	6	—	—	—	—	—	+
	7	+	—	+	—	—	+
	8	—	+	—	—	—	+
C. 23 cm.	9	+	+	+	+	+	+
	10	+	+	+	+	+	+
	11	+	+	+	+	+	+
	12	+	+	+	+	+	+

By consulting this table it becomes apparent that exposures at a distance of 23 centimeters from the lamp were entirely ineffective, but that the amœbæ exposed at distances of 10 and 15 centimeters were invariably killed in forty seconds, and usually killed in ten seconds, although an occasional positive result was obtained even after exposures of thirty seconds. However, these failures may readily be explained. The growth of amœbæ and associated bacteria on plates at the end of twenty-four hours is dense, forming a very perceptible layer on the surface of the agar. It is well known that the ultra-violet rays of light have no greater power of penetration. Therefore some of the amœbæ in a few of the exposures have survived owing to the absorption of the rays by the superficial layers of the growth. This could not occur in water passing through a sterilizer operated by ultra-violet rays.

Since all of these plates contained many encysted amœbæ, and since positive cultures could never be obtained from those plates exposed forty seconds or more at 10 and 15 centimeters, and rarely after ten-second exposures, we are justified in concluding from this experiment that encysted amœbæ are killed on solid media by ultra-violet radiation. The subject of encysted amœbæ will be further discussed in Experiment 7, where a fluid medium was used.

The effect of ultra-violet rays upon amœbæ in a fluid medium was now tested. These experiments are considered to be of much more value than the preceding ones, because they approximated the conditions which would obtain in the practical sterilization of water by the apparatus above described. The results are shown in the following 5 experiments.

Experiment 3.—A number of ordinary hollow-ground slides were sterilized and a large drop from the stock culture of amœbæ was placed in the hollow chamber of the slide. A culture was then made from this drop by transferring a small loopful from the slide to a test tube containing weak sterile bouillon. As soon as the culture was made, the slide was placed under the center of the lamp and the current turned on for a few seconds. After the exposure to the ultra-violet rays a second culture was made in a similar manner from the drop on the slide. Table III shows the results of such an experiment.

TABLE III.—*Experiment 3: Result of cultures from bouillon inoculated with amœbæ. Cultures made before and after exposure to ultra-violet rays.*

No.	10 cm. from lamp.			15 cm. from lamp.		
	Culture before exposure.	Exposure in seconds.	Culture after exposure.	Culture before exposure.	Exposure in seconds.	Culture after exposure.
1	+	5	—	+	5	+
2	+	5	—	+	5	—
3	+	5	—	+	5	+
4	+	10	—	+	10	—
5	+	10	—	+	10	—
6	+	10	—	+	10	—
7	+	20	—	+	20	—
8	+	20	—	+	20	—
9	+	20	—	+	20	—
10	+	30	—	+	30	—
11	+	30	—	+	30	—
12	+	30	—	+	30	—

It will be seen that in this experiment the amœbæ were killed in every trial at 10 centimeters from the lamp and were also killed in all exposures of ten seconds or more at a distance of 15 centimeters. This experiment was particularly satisfactory although it must be admitted that all circumstances were very favorable, since the amœbæ were in a thin layer of clear fluid.

Experiment 4.—This was performed in a manner similar to experiment 3. Distilled water was placed in ordinary staining dishes in sufficient quantity to form a layer 2.5 centimeters deep. This water was heavily inoculated with amœbæ from the stock culture. A loopful of this water was cultivated in a test tube of dilute bouillon in order to prove the presence of amœbæ, the dish of water was then exposed to the ultra-violet rays, and a second culture at once made to show the effect of the exposure. The results of this experiment are shown in Table IV.

TABLE IV.—*Experiment 4: Result of cultures from water inoculated with amœbæ. Cultures made before and after exposure to ultra-violet rays; distance from lamp, 10 centimeters.*

No.	Culture before exposure.	Exposure in seconds.	Culture after exposure.
1	+	5	—
2	+	5	—
3	+	5	—
4	+	5	+
5	+	10	—
6	+	10	—
7	+	10	—
8	+	10	—
9	+	20	—
10	+	20	—
11	+	20	—
12	+	20	—
13	+	30	—
14	+	30	—
15	+	30	—
16	+	30	—

This experiment was equally satisfactory in demonstrating that motile amœbæ can readily be killed by exposure to ultra-violet rays. In order to clinch the matter two more experiments were performed.

Experiment 5.—Distilled water was inoculated with amœbæ, and exposed in the same manner as in the previous experiment, but instead of merely taking a loopful for cultivation after the exposure, the entire amount of water exposed was poured into a small flask containing bouillon, and this flask was examined for amœbæ after several days' cultivation.

Experiment 6.—This was the same as the previous experiment except that instead of distilled water, muddy water taken from the Pasig River was used. This was done in order to determine whether amœbæ would be killed in cloudy water or whether the rays would be intercepted by the particles in suspension, thus permitting some amœbæ to escape. For controls in both of these experiments a dish of the water used was inoculated and without exposure was at once poured into a flask of bouillon. These two experiments are shown in Table V.

TABLE V.—*Experiments 5 and 6: Results when clear and muddy waters were inoculated with amœbæ, exposed to ultra-violet rays and the entire amount of water poured into flasks containing bouillon; distance from lamp, 10 centimeters.*

No.	Experiment 5, clear water.		Experiment 6, muddy water.	
	Exposure in seconds.	Culture in flask.	Exposure in seconds.	Culture in flask.
1	10	—	20	—
2	10	—	20	—
3	20	—	20	—
4	20	—	30	—
5	30	—	30	—
6	30	—	30	—
7	Control not exposed.	+	Control not exposed.	+

From Table V it is evident that amœbæ were killed by the ultra-violet rays in both clear and muddy water after ten-seconds' exposure in the former case and after twenty-seconds' in the latter. Another very interesting and important point was noticed in experiment 6. The control flask that received the Pasig River water inoculated with amœbæ not only developed many amœbæ after a few days' cultivation, but also a large number of balantidia of unknown species. No balantidia were found in any of the flasks containing water which had been exposed to the ultra-violet rays. This affords very good evidence that balantidia as well as amœbæ are killed by ultra-violet rays. These balantidia are more or less constantly present in the Manila water supply and are probably harmless, but *Balantidium coli* is recognized as a dangerous parasite.

It will be remembered that in experiment 2 the encysted amœbæ on solid medium were not destroyed invariably by exposures of less than forty seconds. In order to demonstrate conclusively that encysted amœbæ may be killed by short exposures to ultra-violet radiations the following experiment was undertaken with a fluid medium, thus avoiding the possibility that some organisms were protected from the rays by the thick overlying film of amœbæ and bacteria.

Experiment 7.—Amœbæ from the stock culture were grown on an agar plate for forty-eight hours, at the end of which time microscopical examination showed very many encysted forms. These forms were as numerous as the active organisms. A little distilled water was poured upon the plate and the amœbæ scraped from the agar, thus forming a suspension containing immense numbers of amœbæ, both encysted and motile. A little distilled water was then placed in a shallow dish, inoculated with one large loopful of the suspension of amœbæ, exposed to the rays and immediately thereafter poured into a flask containing weak sterile bouillon. After all inoculations and exposures had been completed the suspension of amœbæ was again examined microscopically and the presence of large numbers of encysted amœbæ demonstrated, thus proving that the encysted forms had not changed into motile forms during the course of the experiment.

The flasks into which the exposed amœbæ had been poured were incubated for forty-eight hours and then examined for the presence of the organisms. The results are shown in Table VI.

TABLE VI.—*Experiment 7: Clear water inoculated with encysted amœbæ, exposed to ultra-violet rays, and the entire amount of water poured into flasks of bouillon; distance from lamp 10 centimeters.*

No.	Time of exposure in seconds.				Controls not exposed.
	5	10	20	30	
1	—	—	—	—	+
2	—	—	—	—	+
3	—	—	—	—	
4	—	—	—	—	
5	—	—	—	—	

This experiment conclusively proves that encysted amœbæ are killed by exposure to the ultra-violet rays for a period as short as five seconds.

CONCLUSIONS.

These experiments taken as a whole undoubtedly demonstrate that in a water supply the amœbæ, whether motile or encysted, may be killed by a comparatively short exposure to ultra-violet rays. Balantidia, also, appear to be destroyed by the same agency. These facts afford a very potent argument in favor of the use of these radiations in the practical sterilization of water in the Tropics. We have had no opportunity to test the apparatus manufactured for this purpose, but since the results of these preliminary experiments have proved so satisfactory we hope to perform this important work in the near future with a view to determining whether the rays are fatal to amœbæ and other protozoal parasites under the conditions obtaining with the commercial sterilizer in practical use.

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A SECOND CONTRIBUTION TO THE ETIOLOGY OF BERIBERI.[1]

By Weston P. Chamberlain and Edward B. Vedder.[2]
(From United States Army Board for Study of Tropical Diseases.)

Our previous paper on this subject(1) entitled A Contribution to the Etiology of Beriberi, as well as the present communication, deal exclusively with polyneuritis of fowls. Therefore it is fitting to state, as an explanation of our title, that we believe that polyneuritis gallinarum and beriberi are essentially the same disease. The identity of two diseases may be considered from several standpoints, including their etiology, pathology, and the symptom complex presented. The causes of beriberi and polyneuritis gallinarum are apparently identical, both diseases being produced by a deficiency of the same as yet unknown substance in the food, and the pathology and symptom complex of the two conditions are practically the same with the exception of the fact that œdema is commonly observed in beriberi and only rarely found in the multiple neuritis of fowls. The similarity is so striking that it is hard to avoid the conclusion that the two conditions are due to the same pathological process causing slightly different manifestations in diverse species. We should expect that two species, varying as widely as man and the domestic fowl in their anatomy and physiology, would react very differently when subjected to the same unfavorable diet of polished rice. The surprising thing, therefore, is not that there are differences in the symptomatology of beriberi and polyneuritis gallinarum, but that the similarity is as great as it is.

In a paper by Chamberlain, Bloombergh and Kilbourne(2) it was shown that in some cases polyneuritis gallinarum could be produced in fowls by prolonged starvation. This observation, however, does not conflict with the statement that the etiology of beriberi and polyneuritis gallinarum are apparently the same. If a fowl is starved completely it is certainly deprived of the neuritis-preventing substance present in food. In such an experiment, therefore, it simply depends upon individual idiosyncrasy whether the fowl will die of starvation before neuritis can develop or vice versa. In other words it is a question whether the bird will die of general starvation or develop neuritis as a result of the absence of certain food elements necessary to nerve nutrition, a phenomenon which we will term partial starvation. In the great majority of cases the fowl will die without nerve degeneration as we should expect, but there will be a few exceptions to this rule for the following reason. Cocks fed on a diet of polished rice contract neuritis in an average of thirty days. In some instances a much longer time is required, while on the other hand many birds develop neuritis in less than thirty days. Several fowls in our experiments have contracted neuritis in nineteen days. Cocks that have been given nothing but water have lived from fourteen to twenty-three days. Therefore, it is apparent that in a few cases it may be possible for the nerve degeneration resulting from partial starvation to occur before the fowl succumbs to general starvation.

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[1] Published with permission of the Chief Surgeon, Philippines Division.

[2] W. P. Chamberlain, major, Medical Corps, United States Army and Edward B. Vedder, captain, Medical Corps, United States Army, members of the United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands.

So far as we know, nothing at all resembling beriberi has ever developed in a professional faster or among men who have undergone starvation. But it has been shown by Frazer and Stanton(3) that it is necessary for men to subsist at least eighty-seven days on a diet of polished rice before cases of beriberi begin to appear, and it is extremely improbable that any man could live eighty-seven days without food. In the case of the fowl, therefore, the incubation period of polyneuritis is such that in a few instances it may fall within the time the bird can resist starvation, a condition that is impossible in the case of man. The observation that certain fowls when starved will develop neuritis is, therefore, no argument against the similarity of beriberi and polyneuritis gallinarum, since there is a perfectly satisfactory explanation for the presence of this phenomenon in fowls and its absence in man.

In our previous communication referred to above we showed that the neuritis of fowls could be prevented by means of an extract of rice polishings containing the following:

	Per cent.
Total solids	1.34
Ash	0.03
Phosphorous pentoxide	0.00165
Nitrogen	0.0406
Sucrose	0.88

We further showed that the neuritis-preventing substance must be soluble in cold alcohol and in cold water, and must be dialyzable. However, we stated that these results were based on a comparatively small series of experiments and that further work would be performed to confirm the observations.

We are now able to state that these results have been fully confirmed. We have prepared this extract on a large number of occasions, and from five different lots of rice polishings. The quantitative analyses of these several extracts have varied slightly from that given above, as might be expected, but there has been no essential difference.

At different times we have fed seven groups, each of which consisted of four fowls, on polished rice combined with this extract in its pure form or after it had been modified by dialysis or by fermentation, with the following results:

Group 1.—Four fowls remained well at the end of seventy days.

Group 2.—Four fowls remained well at the end of seventy days.

Group 3.—Four fowls remained well at the end of one hundred days.

Group 4.—Three fowls remained well at the end of fifty days and one died, apparently of starvation, without evidence of neuritis.

Group 5.—Four fowls remained well at the end of sixty days.

Group 6.—Four fowls remained well at the end of fifty-four days.

Group 7.—Four fowls remained well at the end of thirty-nine days.

This makes a total of twenty-seven fowls that we have maintained in health on a diet of polished rice by the addition of this extract. This same rice repeatedly caused the development of neuritis within thirty days in fowls of our other experiments. Fraser and Stanton report a number of experiments in which the fowls were kept for only thirty-five days, a period within which neuritis ordinarily declares itself, but we have kept our birds from fifty to one hundred days. We have now been studying polyneuritis gallinarum for more than eighteen months and after observing a large number of fowls we think it is quite certain that no group of twenty-seven fowls could be maintained on a diet of polished rice for thirty days without the development of a single case of neuritis, unless they received some neuritis-preventing substance in addition to the rice. When this period is prolonged to fifty, seventy, and one hundred days, the results become conclusive.

Therefore, we regard it as proved that our extract of rice polishings prepared as described in our previous paper contains a neuritis-preventing principle.

By the same reasoning we regard it as proved that this neuritis-preventing substance is dialyzable. The four fowls that were kept one hundred days were fed on polished rice combined with the diffusate obtained from this extract.

In this connection it is interesting to note that after our first paper had gone to press we received the last communication published by Frazer and Stanton(3) in which they showed that the dialysate was incapable of protecting fowls from neuritis, thus independently confirming our observation. They also stated their inability to obtain the diffusate resulting from the process of dialysis owing to its putrefaction. Therefore, it will be interesting to give in detail the method by which we succeeded.

The condensed extract was placed in a parchment bag and suspended in a glass jar containing distilled water in such a manner that the surfaces of the two liquids were on the same level. A few cubic centimeters of chloroform were then added to the bag and also to the water outside the bag, and the whole apparatus was placed in the ice box. The combined action of the cold and the chloroform prevented all decomposition and the dialysate and diffusate were perfectly sweet after ten days of dialysis. The diffusate was removed every two days, and replaced by fresh distilled water, in order that the dialyzable substances might be completely removed from the extract in the bag. These separate portions of the diffusate were subsequently mixed before feeding it to fowls. This method was effectual and enabled us to collect and test the diffusate, proving that it contains the neuritis-preventing principle.

The next step was to consider the action of the sucrose contained in this extract. It was utterly inconceivable on physiologic grounds that sucrose could have the slightest power to prevent neuritis, but since 0.88 out of 1.34 per cent of the total solids consisted of this dialyzable substance, we decided to give it a fair trial by experiment. Two separate experiments were undertaken for this purpose as follows:

Experiment 5.[3]—A quantity of extract was prepared from rice polishings as described previously, and was then inoculated with kitchen yeast and allowed to undergo fermentation in the incubator. Fermentation was very rapid for several days but finally ceased entirely. The extract was then filtered until it was absolutely clear and the microscope showed no yeast cells in the filtrate, in order to avoid adding yeast cells or their subsequent decomposition products to the extract. The alcohol formed by fermentation was then expelled by boiling the filtrate. This was subsequently diluted to its original bulk, reinoculated with yeast, and fermented again in order to make sure that the accumulation of alcohol had not stopped fermentation before all the sugar was exhausted. After assuring ourselves that the sugar was all removed from the extract by fermentation, the yeast cells were again carefully filtered out.

This extract contained all the substances extracted from rice polishings by this method with the exception of the sucrose,[4] and it was made in such proportion that 10 cubic centimeters of the extract would contain the substances extracted from 10 grams of polishings. A solution was also made containing 2 per cent of chemically pure sucrose in distilled water.

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[3] Experiments one, two, three and four are detailed in our former article the title of which is given in reference one.

[4] The sucrose was undoubtedly removed. It is possible that some other substances also may have been broken up by the action of the yeasts or associated bacteria. We have some reason to think that the neuritis-preventing substance may be decomposed by action of bacteria.

Eight fowls were now fed on polished rice. The first four were given a daily dose of 10 cubic centimeters of the fermented extract, while the second four were given 10 cubic centimeters daily of the solution of sucrose. The result of this experiment was as follows:

Group A: Four fowls fed on polished rice plus 10 cubic centimeters of fermented extract daily.—One fowl died of inanition, after thirty days having refused to eat for several weeks. This fowl did not have neuritis. The three remaining fowls were alive and well at the end of fifty days when the experiment was discontinued.

Group B: Four fowls fed on polished rice plus 10 cubic centimeters of a 2 per cent solution of sucrose.—One died of inanition in twenty-four days without neuritis; one developed neuritis in twenty-six days; one developed neuritis in twenty-eight days; one was alive and well at the end of fifty days when the experiment was discontinued.

Neuritis developed in 2 of the 4 fowls which received sucrose in twenty-six and twenty-eight days respectively, and did not develop during fifty days in any of 4 fowls that received the extract deprived of sucrose. This indicates that the sucrose is of no importance in the prevention of neuritis.

Experiment 6.—This experiment arrived at the same result as regards the inefficiency of sucrose, but in a different manner. An extract of rice polishings was prepared by the method detailed in our previous paper(1), and was slowly filtered through bone black (animal charcoal). As is well known this kind of charcoal adsorbs many substances, but allows practically the entire bulk of the sugar in a solution to pass through. The extract as poured on the bone black was yellow in color, but the filtrate through the bone black was perfectly clear and appeared like water. However, fermentation tests, showed that this filtrate contained practically all of the sucrose present in the original extract.

The bone black remaining on the filter was then transferred to a flask and repeatedly shaken and washed with distilled water in order to remove, if possible, the ingredients separated from the extract by this substance. The attempt was only partially successful, since a clear fluid having a very faint bluish tint was obtained, as compared with the straw-colored fluid originally poured upon the bone black.[5]

Eight fowls were then fed on polished rice. The first 4 were given a daily dose of 10 cubic centimeters of the filtrate through bone black while the second 4 received a daily dose of 10 cubic centimeters of the substances subsequently removed from the bone black by distilled water. The result of this experiment is as follows:

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[5] The bluish tinge is believed to have been due to some impurity in the charcoal.

Group A: Four fowls fed on polished rice plus 10 cubic centimeters daily of the filtrate through bone black.—Two fowls developed neuritis in twenty-two days; one fowl developed neuritis in twenty-four days; one fowl developed neuritis in twenty-six days.

Group B: Four fowls fed on polished rice plus 10 cubic centimeters of the washings of bone black daily.—One fowl developed neuritis in forty-one days; one fowl developed neuritis in forty-nine days; two fowls were alive and well on the fifty-sixth day when the experiment was discontinued.

In this experiment all 4 fowls that received the filtrate containing sucrose developed neuritis. This experiment and experiment 5 show conclusively that sucrose is incapable of preventing polyneuritis gallinarum.

We also have demonstrated in the last experiment that the neuritis-preventing principle is retained in a filter of bone black, and, therefore, bone black must have a strong power of adsorption for this substance. Washing the charcoal with water appears to remove *some* of the neuritis-preventing substance as shown by the fact that 2 out of 4 fowls were completely protected for at least fifty-six days, while in the case of the 2 birds which developed neuritis the disease manifested itself only after an unusually prolonged incubation period. It is probable that the neuritis-preventing principle can be extracted completely from this bone black by using other solvents and that this will afford another new method for the separation and identification of this important substance.

In addition to the experiments with extract of rice polishings just described, we have tested the neuritis-preventing properities of several other articles.

Experiment 7.—It has been suggested several times that beriberi and scurvy are closely allied diseases. This seems quite improbable owing to the vast difference in the pathology and symptomatology of the two conditions. Lime juice is well known to be both a preventive and a cure for scurvy, and if there is anything in the supposition that the two diseases are allied we might reasonably expect that the administration of lime juice would prevent the development of polyneuritis gallinarum. To test this hypothesis 4 fowls were fed on polished rice and were given a daily dose of 0.8 of a cubic centimeter of lime juice diluted with water to 10 cubic centimeters. This quantity for a fowl is equivalent to about 40 cubic centimeters for a man, which is ample to prevent the development of scurvy. The result of this experiment is as follows:

Group A: Four fowls fed on polished rice plus 0.8 cubic centimeter of lime juice daily.—One fowl died of inanition on the twenty-fourth day; one fowl developed neuritis on the twenty-fifth day; one fowl developed neuritis on the thirty-sixth day; one fowl was alive without neuritis, although rather weak on the thirty-ninth day when the experiment was discontinued.

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Two fowls out of 4 developed neuritis while receiving lime juice daily. Therefore, it is apparent, that there is nothing in lime juice which will prevent polyneuritis gallinarum and it seems quite probable that there can be no etiological similarity between scurvy and either polyneuritis gallinarum or beriberi.

It has been suggested by several authors that neuritis of fowls and beriberi are due to a lack of nucleins in the food. The nucleins are combinations of an albumin with nucleic acid, a very complex substance containing a considerable proportion of phosphorus. Our experiments have excluded such a substance from consideration since it would not be dialyzable, and since it has been shown in our former article(1) that phosphorus is immaterial and unnecessary in preventing neuritis. We desired, however, to experiment with nuclein in order that we might have a direct experiment with one of these highly phosphorized proteids.

Experiment 8.—Therefore, four fowls were fed on polished rice and given a daily dose of 0.2 gram of dried nuclein. This is a very large quantity compared with the nuclein that would be naturally received in the food of a fowl. The nuclein was obtained from a leading pharmaceutical house in Manila.

Group A: Four fowls fed on polished rice plus 0.2 gram of nuclein daily.—One fowl developed neuritis in thirty-three days; one fowl developed neuritis in thirty-six days.

Two fowls were well after fifty-six days when the experiment was concluded.

As 2 fowls out of 4 developed neuritis, it is not believed that the nuclein used had any decided power to prevent polyneuritis gallinarum. Since the incubation period for the 2 fowls which did develop neuritis is perhaps slightly above the average, and since 2 fowls remained well at the end of fifty-six days, it can not be denied that there may have been a small amount of neuritis-preventing substance in the nuclein, a quantity sufficient to retard the outset of the disease. As will be stated later on, our work has led us to suspect that the neuritis-preventing principle may ultimately be found among the decomposition products of protein. It is possible that small quantities of such material may have been present in the nuclein. We have not made any further experiments with nuclein because the question as to whether or not nuclein was efficacious had no direct bearing on the main line of investigations we were pursuing.

Hulshoff-Pol(4) proved that a decoction of *katjang.idjo* (*Phaseolus radiatus*) prevented and cured beriberi. It has been generally accepted in the Philippines that these beans, known here under the name of *mongos,* possess this property, and for a while mongos were supplied to the Philippine Scouts (native) as a part of their ration for the purpose of preventing beriberi. However, it was found difficult to obtain mongos in sufficient quantity in the local market, they did not keep very well, and the Scouts did not like them as a steady article of diet. There are no such objections to the use of the ordinary white bean that constitutes a part of the army ration, but there was no information obtainable as to whether it would prevent beriberi. It seemed to us quite probable that the white bean would be just as efficacious in this respect as any other legume. Therefore, we tested these beans in the following experiment.

Experiment 9.—One kilogram of dried white beans was soaked over night in distilled water, and the following day boiled for two hours, allowing the decoction to evaporate down to such a point that 1 liter of fluid remained. The cloudy liquid obtained was filtered until perfectly clear. This fluid was of a deep yellow color with a tendency to become opalescent on standing, and had a distinct odor of beans. It was preserved in the ice box with the addition of a slight quantity of chloroform. Ten cubic centimeters of the fluid represented the substances extracted by boiling water from 10 grams of beans. We then treated 1 kilogram of mongos in a precisely similar manner, obtaining from them a corresponding extract.

Nine fowls were now fed on polished rice. Five fowls received daily in addition 10 cubic centimeters of extract of beans, while the other 4 fowls received 10 cubic centimeters of extract of mongos. The result of this experiment is as follows:

Group A: Five fowls fed on polished rice plus 10 cubic centimeters of extract of beans daily.—All five fowls remained healthy at the end of sixty days when the experiment was discontinued.

Group B: Four fowls fed on polished rice plus 10 cubic centimeters of extract of mongos daily.—One fowl developed neuritis on the thirty-fifth day; the other three fowls remained well at the end of sixty days when the experiment was discontinued.

We do not believe this experiment to indicate that mongos will fail to prevent beriberi. On the contrary, we believe that they will prevent this disease. The extract of mongos as we prepared it was filtered until perfectly clear, and it is quite probable that we removed in this manner many of the substances which were present in Hulshoff-Pol's decoction. This part of the experiment, therefore, only demonstrates that the extract as we prepared it failed to confer complete protection. The important fact in this experiment is that the fowls receiving extract of beans were completely protected. Therefore, the ordinary white bean, must contain a neuritis-preventing principle.

While we do not wish to claim too much on the basis of a single experiment, we ourselves are convinced that the ordinary white bean will prove equally as efficacious as the mongo in the prevention of beriberi. This is a very important observation since it indicates that we may use these beans as a preventive against beriberi in the rations of native troops, native prisoners, and others. Probably in actual practice the ordinary white bean may be even more efficacious than mongos, because beans are more savory and are more desired as an article of diet by all classes of men. This point must be borne in mind when prescribing for natives a diet consisting largely of rice. If the natives do not happen to care for mongos, or the other articles introduced into the ration for the purpose of preventing beriberi, they will not eat them, but will live on an almost exclusive diet of rice; but the man, native or white, who does not relish well cooked beans is hard to find, and it is believed that they would be generally eaten by persons who would refuse to eat mongos.

It is also quite possible that this observation will be of further assistance in identifying the beriberi-preventing principle. Extracts of beans and of rice polishings doubtless contain many substances peculiar to themselves, but we may find some substance, or a few substances, common to both of these extracts. If this should occur, it is probable that the neuritis-preventing principle will be found, or at least the search for it will be restricted to very narrow limits.

We have also performed some experiments in the course of which we evaporated the extract of rice polishings to dryness in a water bath at 100°C. This necessitated prolonged heating at this temperature and we found that the extracts so prepared had lost their efficacy. It has long been known that even a brief exposure to a temperature of 120°C. destroyed the neuritis-preventing substance and it is now shown that prolonged exposure to a temperature of 100°C. will also produce the same effect. It is possible that this is the reason why our decoction of mongos lost some of its power, and we would recommend, therefore, that no extract supposed to contain the neuritis-preventing principle should be boiled for more than one hour.

CONCLUSIONS.

We have made some progress in the identification of the neuritis-preventing substance contained in the extract of rice polishings prepared by the method described in our first paper. Of 1.34 per cent total solids contained in this extract, 0.03 per cent was ash. This we believe to be negligible since it consists entirely of inorganic constituents, chiefly of lime, magnesia, and potassium carbonate. We have tried salts of calcium, magnesium, and potassium and found them wanting. Nitrogenous matter comprises 0.04 per cent of the total solids. Of this only 0.02 per cent was present in the diffusate, which has been shown to contain the neuritis-preventing principle, and the remaining 0.02 per cent can be eliminated because it remained in the dialysate which failed to prevent neuritis. The 0.88 per cent sucrose is of no importance. Combining these unimportant substances and subtracting them from the 1.34

per cent of total solids, we find that there is only 0.4 per cent remaining. The neuritis-preventing principle must, therefore, be sought for in this 0.4 per cent of solid matter and must be a substance that is dialyzable, that is soluble in water, in 95 per cent alcohol, and in 0.3 per cent hydrochloric acid, which is easily decomposed by heat and which possesses a strong affinity for bone black. The same substance or a similar substance is also contained in a decoction of ordinary white beans. Bodies corresponding to this description are found among the decomposition products of the proteids. Therefore, it appears to us, that we have obtained sufficient information with regard to the nature of this body to attempt to identify it by the direct methods of chemical analysis. We have already begun experiments along these lines with the assistance of Mr. R. R. Williams, of the laboratory of organic chemistry, Bureau of Science, Manila, and expect to report them in the near future.

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A STUDY OF ARNETH'S NUCLEAR CLASSIFICATION OF THE NEUTROPHILES IN HEALTHY ADULT MALES AND THE INFLUENCE THEREON OF RACE, COMPLEXION, AND TROPICAL RESIDENCE.[1]

By WESTON P. CHAMBERLAIN and EDWARD B. VEDDER.[2]

(From the United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands.)

INTRODUCTION.

In the quarterly report of the Board for September 30, 1910 (published in the Military Surgeon for February, 1911) there was a preliminary report upon some observations by the Board regarding the Arneth classification of the neutrophiles for Filipinos and for white men living in the Philippines. Since the original work a much larger series of cases has been studied, counts having been completed on 72 Americans and 50 natives. The earlier and the later observations were made independently by different members of the Board and the results of both series are practically identical. Therefore, it is safe to say that the personal equation, which might be a considerable factor in this kind of work, has been discounted in making up the final result. These examinations were originally undertaken as a part of the investigation into the influence of tropical light on blonds and brunettes, and the research was subsequently extended to natives among whom most interesting results have been obtained. As far as we can learn no work along this line has previously been undertaken in the Tropics.

BRIEF DESCRIPTION OF ARNETH'S CLASSIFICATION.

A classification of polymorphonuclear neutrophiles based on the number of nuclei or nuclear fragments was proposed by Arneth in 1904.(1) He described

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[2] Weston P. Chamberlain, major, Medical Corps, United States Army and Edward B. Vedder, captain, Medical Corps, United States Army, members of the United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands.

5 main classes.[8] Class I has a single nucleus which may be round or of irregular shape. If the nucleus is round, the cell is then identical with Ehrlich's neutrophilic myelocyte(3) which is not found normally in the peripheral circulation. Class II includes the neutrophiles with 2 nuclei or nuclear fragments. Class III has 3 nuclei or fragments and is the largest class in normal blood. Classes IV and V have respectively 4 and 5 nuclei or nuclear fragments. A fairly constant proportion between the different classes is found in normal blood. The "neutrophilic blood picture" as given by Arneth is for each one 100 polymorphonuclear leucocytes as follows:

Class I.	Class II.	Class III.	Class IV.	Class V.	Index (Arneth).	Index (Bushnell and Treuholtz).
5	35	41	17	2	40.0	60.5

Simon(3) gives the following normal range for each 100 neutrophiles:

Class I.	Class II.	Class III.	Class IV.	Class V.
4–9	21–47	33–48	9–23	2–4

The so-called nuclear fragments seldom if ever represent separate nuclei, but only lobes of a polymorphous nucleus, the connecting nuclear substance being drawn out into a fine thread. The "index" is a standard for comparison of different pictures. Arneth adopted as an "index" the sum of classes I and II while Bushnell and Treuholtz selected the sum of classes I and II and one-half of class III.(13)(14)

The polymorphonuclear leucocyte is the active phagocytic cell of the blood stream and the corpuscles with 3 or 4 nuclear fragments are considered the adults and are thought to be most active as phagocytes and best fitted to protect the body against invading organisms. The superannuated cells represented by class V and the immature cells represented by classes I and II are less able to take up the defence of the body. Pottenger in a series of experiments found a gradual rise in phagocytic power from class I to class IV inclusive, and a decrease in class V.

When the first and second classes are increased above normal and the third and fourth are correspondingly decreased the condition is spoken of as a "shift or a drift to the left" while the reverse alteration is called a "shift to the right." Pottenger,(4) Klebs,(15) Kagan,(2) Minor and Ringer(13) and others have found a decided shift to the left in patients with marked lesions of tuberculosis and Kagan also found a less marked shift to the left in cases of sepsis. Solis-Cohen and Strickler were not able to find any shift to the left in tuberculous patients at any stage of the disease although the polymorphonuclear cells were reduced in numbers.(6) Arneth considers a shift to the left an evidence of lowered resistance to the disease affecting the patient whose blood he is studying, as is indicated by the fact that when a tubercular patient improves the blood picture tends to return to the normal.(13)

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[8] Arneth subdivided his 5 main classes into a number of smaller groups with distinctions depending upon the indentations and the character of the loops and lobes. These sub-groups seem to us to be an unnecessary and impractical refinement and moreover the number in each sub-division is too small to be of value unless 500 or 1,000 neutrophiles are counted(6). Such a procedure is very time-consuming and does not appear to us to promise compensating advantages.

OUR METHOD OF STAINING AND COUNTING.

Most of our counts were made with very thin smears stained with Wright's stain. A few were stained with hematoxylin and eosin after the method of Bushnell and Treuholtz. This method gave excellent pictures but did not seem to offer sufficient advantages to compensate for the trouble of making a second stain for the differential count. Whatever staining method is used, it is important that smears be thin. We have tried the technique of Wiedenreich, but without success.(16)

Even in a well-spread and well-stained slide there will always be an uncertainty as to the group in which some nuclei should be classified. Pottenger considers that these doubtful cells constitute 12 per cent of the total when Wright's stain is used. To obtain uniform results, we adopted the following rules in counting:

(1) Nuclear masses connected by a distinct isthmus were counted as 1 nucleus, while masses connected by only a thread were counted as 2 nuclei.

(2) Nuclear masses clearly superimposed were considered as separate nuclei, but where the superposition was not distinct the body was considered a single nucleus.

(3) In all instances when there was doubt as to whether a cell should be grouped in one or the other of two classes, it was always placed in the *higher* class.

By a careful adherence to rule 3 we have avoided the possibility of producing an artificial shift to the left. If all doubtful cells had been recorded alternately, the first in a lower and the second in a higher class, the shift to the left which we found for bloods in the Tropics would have been even more pronounced than we now claim.

In our work we enumerated for differential counts 200 cells for each individual. For the Arneth work we counted 200 cells each from a part of the men and 100 each from the remainder. While an enumeration of 100 cells for Arneth work and of 200 cells for differential counts may in a few instances lead to slight errors for the individual, it is believed that it is ample to give accurate average results such as we were seeking in our investigation.

The following table indicates how close an agreement in the results for both differential and Arneth counts was obtained by our two independent observers working at different times.

TABLE I.—*Agreement of differential and Arneth counts as made by two independent observers in the Philippines.*

Observer.	Men observed.		Differential counts, per cent.						Arneth's classification, per cent.					Index (Arneth).	Index (Bushnell and Treuholtz).
	Race.	Number.	Polynuclears.	Eosinophiles.	Small lymphocytes.	Large lymphocytes.	Transitionals.	Mast cells.	I.	II.	III.	IV.	V.		
A	Americans	22	56.4	5.1	30.0	4.1	3.5	0.9	13.4	34.8	39.0	11.3	1.5	48.2	67.7
B	Americans	50	57.0	3.2	32.4	5.0	1.7	0.7	13.2	32.0	36.3	17.3	2.1	45.2	63.3
A	Filipinos	21	48.1	13.6	28.1	6.2	3.2	0.8	28.1	41.2	24.7	5.3	0.7	69.8	81.6
B	Filipinos	29	55.1	8.6	31.2	3.4	1.1	0.6	27.0	36.3	26.6	9.1	1.0	63.8	76.6

It will be observed that the Arneth index of Observer A is slightly higher for Americans than that of Observer B and that the same is true for the observations on Filipinos, so there is complete agreement between the two observers as regards the finding of a marked shift to the left for natives. The same remarks are true when the Arneth counts are compared on the basis of the index of Bushnell and Treuholtz.

THE INFLUENCE OF RACE AND TROPICAL RESIDENCE ON THE DIFFERENTIAL AND THE ARNETH COUNT.

The appended tables (Nos. V and VI) give the individual data for our series of 122 counts. When divided according to race, American or Filipino, the white cell count per cubic millimeter was found within normal limits in both races,(7) averaging a little higher for the Filipinos, 9,248 as compared with 7,304 for Americans. The average differential leucocyte count showed for both races the characteristic changes found by this Board,(9) (10) and by others,(11) (12) in the blood of Filipinos and of white men resident in the Philippines, namely, a decreased polymorphonuclear count associated with an increased percentage of small lymphocytes and, for the natives, an increase in the eosinophilic cells due in the vast majority of cases to infestation with intestinal parasites. The reduction in polymorphonuclear neutrophiles was slightly greater for natives, who had 52 per cent of these cells as compared with 56 per cent for Americans.

When the Arneth count came to be considered very marked differences were found between the averages for the two races. There was evident a slight shift to the left in the average count for white men as compared with the standard recognized by most authorities in Europe and America.[4] Since we have not counted for comparison any extensive series of blood smears from men residing in America, we are unable to state whether this slight drift to the left is due to climatic influence or to our own personal equations in enumerating. For the Filipinos we found a very decided shift to the left and are sure that this represented an actual and wide departure from the Caucasian average, because we have as a standard for comparison our series made simultaneously on white men in the same locality.

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[4] These normal "standards" vary considerably with the writer, and we believe this variation is in part due to the fact that most observers have counted too small a series of normal cases.

Our average results for differential and Arneth counts for the two races, compared with several writers' estimates of the normal are shown in Table II. For the 72 Americans the average length of the *present tour* of tropical duty was 14.6 months and the average total tropical service 28.7 months.

TABLE II.—*Arneth counts on healthy Americans in the Philippines and on healthy Filipinos, contrasted with normal counts in Europe and America.*

	White cells per cu. mm.	Differential counts, per cent.					Arneth's classification, per cent.					Index (Arneth).	Index (Bushnell and Treuholtz).
		Polynuclears.	Eosinophiles.	Small lymphocytes.	Large lymphocytes.[a]	Mast cells.	I.	II.	III.	IV.	V.		
Normal (Simon)	3,000 to 10,000	60 to 70	1 to 4	20 to 30	1 to 6	0.2 to 1.0	4 to 9	21 to 47	33 to 48	9 to 23	2 to 4	25 to 56	------
Normal in Europe (Arneth)	------	------	------	------	------	------	5	35	41	17	2	40.0	60.5
Normal in United States (Kagan)	------	------	------	------	------	------	5	19	46	25	5	24.0	47.0
Normal (Buchanan and Williams)[b]	5,000 to 10,000	70 to 90	0.5 to 4	20 to 30	3 to 6	[b]0.5	8	36	42	13	[b]1	44.0	65.0
Average for 72 healthy Americans in Philippine Islands	7,304	56.8	3.8	31.7	6.9	0.8	13.3	32.9	37.2	14.6	2.0	46.2	64.8
Average for 50 healthy Filipinos	9,248	52.2	10.6	29.9	6.6	0.7	27.5	38.3	25.8	7.5	0.9	65.8	78.7

[a] With the large lymphocytes have been classed the transitional forms, following the grouping of Simon.

[b] The average white and differential counts given in this line are from R. J. M. Buchanan, Blood in Health and Disease (1909). The Arneth averages are from 100 counts on 55 individuals in the United States by W. W. Williams in *Colorado Medicine* (1911), 8, 175.

From the tables it is evident that there is a very marked shift to the left in the Arneth count for Filipinos and apparently a slight movement in the same direction for Americans resident in the Philippine Islands.

THE ARNETH INDEX IN THE TWO RACES.

The index, referred to in an earlier part of this paper, is very useful to give at a glance a basis of comparison between different Arneth counts.

The more marked the shift to the left, the higher will be the index. For our American series the index adopted by Arneth (sum of classes I and II) was 46.2 which is but a little higher than the index of 40 found by Arneth for normal individuals in Europe. For the Filipinos of our series the index is much elevated, averaging 65.8.

Using the index adopted by Bushnell and Treuholtz (sum of classes I and II and one-half of class III) there also appears a decided elevation for the Filipinos, average 78.7, as compared with 64.8 for Americans resident in the Philippines, but the differences are not as great as when the index adopted by Arneth is employed.

THE INFLUENCE OF THE COMPLEXION OF WHITE MEN ON THEIR ARNETH COUNT IN THE PHILIPPINES.

We have for eighteen months been at work on the influence of the Philippine climate on soldiers of the blond and the brunette types of complexion who have served a year or more in the Islands. By blond we mean a man with light brown, sandy, red, or flaxen hair, blue or gray eyes, and a light or ruddy complexion; by brunette one with dark brown or black hair, brown or black eyes, and a dark complexion. Men in whom eyes, hair, and complexion do not *all* conform to the same type are classed as mixed types and excluded from consideration. The blood counts considered below are from men who were pronounced specimens of the type they represent.

TABLE III.—*Leucocyte count, differential count and Arneth's count on 28 blonds and 28 brunettes resident in the Philippines.*

Complexion type.	White cells per cubic millimiter.	Differential counts, per cent.						Arneth's classification, per cent.					Index (Arneth).	Index (Bushnell and Treuholtz).
		Polynuclears.	Eosinophiles.	Small lymphocytes.	Large lymphocytes.	Transitionals.	Mast cells.	I.	II.	III.	IV.	V.		
Blond	7,077	55.5	4.3	33.0	4.5	1.9	0.8	11.5	31.6	38.2	16.2	2.1	43.1	62.2
Brunette	7,888	60.2	3.5	29.0	4.5	2.1	0.7	14.5	34.5	35.7	13.6	1.8	49.0	66.8

The length of the present tour of tropical service averaged 16.5 months for the blonds and 16.2 for the brunettes. If previous tours of tropical service are added then the average total tropical service was 29.1 months for the blonds and 36.0 months for the brunettes.

It will be observed that the proportion of the polymorphonuclear leucocytes (phagocytic cells) is slightly less for the blonds (55.5 per cent as compared with 60.2), while the brunettes show a somewhat higher index for the Arneth count, whether reckoned by the method of Arneth or that of Bushnell and Treuholtz. We believe that these differences are unimportant and due to the inherent error attaching to such a small series of cases. Some authors have considered that blonds are less well able to stand the influence of a tropical climate. As far as our small series of observations is concerned, we do not think this view is supported; in fact the higher Arneth indexes for brunettes, if found to be constant in larger series, might indicate the reverse condition.

THE POSSIBLE SIGNIFICANCE OF LOW POLYMORPHONUCLEAR COUNTS AND HIGH ARNETH INDEXES IN FILIPINOS.

In estimating the significance of the blood counts which we have found in Filipinos it is necessary to consider the four factors discussed below.

1. *Low polymorphonuclear count in Filipinos.*—That the polymorphonuclear cells of Filipinos and of Americans long resident in the Philippines are reduced below the minimum point considered normal for Americans and Europeans at home seems quite well settled. Some results found in healthy persons in the Philippines are shown in the following table.

TABLE IV.—*Reduction in polymorphonuclear neutrophiles found in the Philippines.*

Observers.	Race.	Number observed.	Residences in Philippine Islands.	Polynuclears.	Eosinophiles.	Small lymphocytes.	Large lymphocytes.[e]	Mast cells.
Wickline (12)	Americans[a]	104	Batangas	54.9	5.1	33.4	6.1	0.5
Guerrero and Sevilla (11)	Filipinos	129	Taytay	51.6	11.2	34.5	4.1	0.2
This Board (10)	Americans[b]	115	Various	58.7	3.6	32.6	4.6	0.5
This Board (9)	Igorots[c]	40	Baguio	46.9	8.9	37.2	5.7	0.4
This Board	Americans[d]	72	Various	56.8	3.8	31.7	6.9	0.8
Do	Filipinos[c]	50	do	52.2	10.6	29.9	6.6	0.7

[a] These were all soldiers who had served continuously in the Philippines over 18 months at the time the counts were made.

[b] All the members of this group were soldiers who had been in the Philippines over a year.

[c] All of this group were adult males.

[d] All of these were soldiers with an average of 14 months' continuous Philippine service.

[e] Includes transitional forms.

In all of the counts made by ourselves care has been taken to count back and forth completely across the slide to avoid obtaining an undue number of small lymphocytes which are liable to be more numerous in the central portion of the smear than at the borders.

That these low polymorphonuclear and high lymphocyte counts for Americans developed gradually after residence in the Philippines and were not present on their arrival in the Islands has been shown by Wickline.(12)

2. *The cells of the body which are phagocytic.*—It is generally accepted that the small lymphocytes have no phagocytic power. Buchanan says that the

"coarse eosinophiles are actively amoeboid and to a certain extent phagocytic"(7), while Kanthack and Hardy consider that they never act in this way.(8) Wesbrook once observed phagocytosis by these cells, but considers it extremely rare.(8) It has also been stated that virulent living bacteria act in a negatively chemiotactic manner on eosinophiles.(5) Buchanan considers the large mononuclears to act as phagocytes,(7) but Kanthack and Hardy believe that only in case of feebly virulent bacteria are they capable of immediate action. That certain fixed cells, notably endothelial cells, have phagocytic power is generally accepted, but the extent and manner of their action is not very thoroughly understood.

As far as the blood is concerned the chief phagocytic cell is the polymorphonuclear neutrophile, the "microphage" of Metchnikoff. This is the only blood cell which is generally recognized as being able to ingulf and destroy bacteria. Its importance in the production of immunity was overshadowed for a time by the work of Ehrlich, but has again been brought into prominence by the researches of Wright.

Cabot says, *"It would appear that the degree of health in persons not organically diseased might perhaps prove to vary directly with the percentage of polymorphonuclear cells in the blood."*

3. *Influence on phagocytosis of a high Arneth index.*—As before stated, Arneth considers that classes I and II of his classification represent the immature leucocytes and that they are less able to protect the body than are the cells with three or four nuclear fragments. Pottenger(4) reports that the phagocytic power of the leucocytes for staphylococci gradually rises from class I to class IV inclusive and diminishes for class V. On the other hand, Buchanan, using cocci, could not detect any relation between the number of nuclear divisions and the number of bacteria engulfed by the cell.

Turning from experimental researches to clinical observations, it will be found that nearly all of the work with the Arneth classification has been done on patients suffering from tuberculosis. It seems quite generally accepted that a marked shift to the left indicates lowered resistance to that disease. In other words, a high Arneth index goes hand in hand with a low resistance or with a high degree of toxic and bacterial absorption which is leading to the destruction of the actively phagocytic cells (classes III and IV). By a large number of examinations in various infectious diseases, Arneth has demonstrated a direct relationship between the blood picture and the course of the disease. The picture is therefore considered an index of the protective efforts of the body against infections.(15)

It seems to us reasonable to conclude from the foregoing that a bad Arneth blood picture, if found habitually in the apparently normal individuals of a race, probably indicates a diminished resistance on the part of that race to various infections. This conclusion is merely offered as an hypothesis.

4. *Leucocytometry in the Filipinos.*—We have not been able to find

much evidence as to the number of white cells per cubic millimeter in the blood of healthy natives. Our own work on 29 adult male Filipinos gave an average count of 9,248. This may seem a trifle high, but it is at least well within the normal upper limit of 10,000 given by Simon, Buchanan and Cabot.

It may be mentioned that our average of 9,248 agrees closely with the average of 9,000 given by Castellani and Chalmers for adult male Bengalese in India.

Summary.—After a consideration of the above four sections it will be evident that in our series of Filipino bloods there is: First, an absolute number of white cells within normal limits; second, a markedly low relative proportion of polymorphonuclear neutrophiles; and third, an abnormally high percentage of the neutrophilic elements which fall in classes I and II of Arneth and which are supposed to be deficient in phagocytic power. Therefore, in the Filipino blood there is both a relative and an absolute reduction in the phagocytes, the cells which, with the aid of opsonins, are concerned in destroying bacterial invaders.

If this state of things is general in tropical races, it may be a visible indication of the lowered resistance of such peoples to certain newly introduced maladies and to some epidemic tropical diseases which generally cause a higher mortality among natives than is experienced among Caucasians. In the first class of diseases may be mentioned measles, leprosy, syphilis, and tuberculosis, and in the second class plague and cholera. Apparently the natives of the Philippines have a good resistance to infections with staphylococci and streptococci, and this clinical fact may be related to the observations of Buchanan who could find no relationship between the degree of nuclear subdivision and the number of *cocci* engulfed by the neutrophilic cells.

POSSIBLE INFLUENCE OF TROPICAL CLIMATE.

Whether the reduction we have found in the phagocytic elements of the blood may be the result of a tropical climate *per se* is an interesting subject for speculation and for future study. About a year ago we suggested(9) that the low polymorphonuclear count in natives and white men in the Philippines might indicate lowered resistance and be due to tropical conditions. The work of Wickline showing that the decrease of polymorphonuclear elements becomes progressively more marked as the length of residence in the Philippines increases, is suggestive that the change is due to the climate. Our recent work with the Arneth classification points in the same direction, since the index for white men who had lived over a year in the Islands is a little higher than has been found normal in temperate climates by most observers. On account of the many complicating factors, three of which are mentioned below, it will be extremely difficult to establish a direct relationship between climate and diminished phagocytic power.

In the case of the Filipinos in our series a possible influence of tuberculosis should be borne in mind. All of the counts were made on apparently healthy laborers, but no physical examinations were made to exclude latent or incipient tuberculosis as a cause of a high Arneth index. Although tuberculosis is widespread among the Filipinos, we do not believe that it is so prevalent as markedly to raise the average Arneth index of a series of 50 men engaged in daily labor and to all outward appearances in perfect health.

Intestinal parasites greatly modify the blood findings in Filipinos and are responsible for the eosinophilia almost invariably met with. It is impossible at present to state whether infestation with intestinal worms produces a modification in the Arneth index.

Diet is a third factor which conceivably might influence a blood picture. The food of the Filipino consists largely of rice and is low in nitrogenous components. It would be of much interest to make a series of Arneth counts on Japanese and northern Chinese, people who dwell in a temperate climate yet have dietary habits similar to those of tropical races.

CONCLUSIONS.

1. Both Filipinos and Americans residing more than a year in the Philippines had a normal average number of white cells per cubic millimeter.

2. In both races the percentage of polymorphonuclear neutrophiles was much decreased below the minimum considered normal for white men in temperate regions.

3. Probably the polymorphonuclear neutrophiles are the only actively phagocytic cells in the circulating blood.

4. The average Arneth picture showed a marked shift to the left in the case of Filipinos and a slight drift in the same direction for Americans resident more than a year in the Philippines.

5. A shift to the left in the Arneth count probably indicates a diminution in the phagocytic power of the blood in question.

6. From the first five conclusions it will be apparent that the Filipinos show an actual absolute reduction in the number of polymorphonuclear neutrophiles (phagocytes), and that of this reduced number an abnormally large proportion are deficient in phagocytic power. In other words, the Filipino has absolutely fewer efficient phagocytes than are found among white men either in the Philippines or at home.

7. This reduction in circulating phagocytic cells may be a visible indication of a lowered resistance to infections on the part of native races.

8. No material differences in the differential count or the Arneth picture were observed between two groups of American soldiers, one group consisting of 28 pronounced blonds and the other group of 28 pronounced brunettes.

TABLE V.—*Differential and Arneth counts on the blood of Americans residing in the Philippines.*

No.	Birthplace.	Residence.	Age.	Tropical service, months.		Complexion.[a]	Red cells.	Hb.	White cells.	Polynuclears.	Large lymphocytes.	Transitionals.	Small lymphocytes.	Eosinophiles.	Mast cell.	Arneth classification.				
				Present tour.	Total.											I.	II.	III.	IV.	V.
								P. ct.												
1	New York	New York	24	12	12	M.	------------	90	6,700	40.0	6.0	4.0	48.5	1.5	0.0	7.5	38.5	37.5	15.0	1.5
2	Pennsylvania	Pennsylvania	33	22	52	M.	------------	95	5,600	43.0	4.0	2.0	48.0	2.0	1.0	9.0	28.0	48.0	13.0	2.0
3	New York	Pennsylvania	32	6	36	Bl.	------------	98	6,300	60.0	6.5	2.0	29.5	1.0	1.0	8.5	31.0	39.5	19.0	2.0
4	Alabama	Georgia	28	13	13	M.	------------	98	6,800	49.0	6.5	3.0	38.0	3.0	0.5	10.0	33.0	36.5	19.5	1.0
5	Missouri	Arkansas	25	11	11	Br.	------------	92	8,500	58.0	7.0	1.5	30.0	3.0	0.5	19.5	35.0	35.5	9.5	0.5
6	Minnesota	Minnesota	23	1	1	Br.	------------	90	8,200	56.7	7.3	1.0	34.0	0.0	1.0	29.0	35.0	27.0	9.0	0.0
7	New York	Nebraska	45	2	13	Br.	------------	98	9,200	67.0	4.0	2.5	23.5	3.0	0.0	18.5	37.5	35.5	8.5	0.0
8	Kansas	District of Columbia.	25	1	3	Bl.	------------	85	5,600	60.0	4.0	2.0	32.0	0.5	1.5	11.5	31.5	40.0	16.0	1.0
9	Pennsylvania	Pennsylvania	32	1	1	Br.	------------	97	9,000	58.0	3.0	2.0	35.5	1.5	0.0	11.5	29.5	36.0	18.0	5.0
10	Missouri	Missouri	25	2	2	Bl.	------------	90	6,400	45.5	7.0	5.5	40.5	0.5	1.0	32.0	28.0	34.0	4.0	2.0
11	Missouri	Missouri	22	16	16	Bl.	4,760,000	95	5,500	44.0	4.5	3.5	41.5	6.5	0.0	11.0	23.0	44.0	20.0	2.0
12	Montana	Montana	23	18	18	Bl.	4,200,000	83	8,200	55.5	1.5	0.5	35.5	6.0	1.0	12.0	25.0	42.0	19.0	2.0
13	Indiana	Nebraska	32	17	17	Br.	5,000,000	92	6,900	64.5	5.0	1.5	27.5	1.0	0.5	12.0	34.0	39.0	14.0	1.0
14	Illinois	Illinois	24	18	18	Bl.	4,200,000	80	6,400	59.0	5.0	2.5	33.0	0.5	0.0	5.0	19.0	44.0	28.0	4.0
15	Texas	Texas	26	18	18	Br.	4,900,000	95	8,200	55.0	4.5	1.5	30.0	8.0	1.0	7.0	25.0	40.0	24.0	4.0
16	Wisconsin	Nebraska	39	16	49	Bl.	4,640,000	85	8,700	70.5	5.0	1.5	19.5	1.5	2.0	9.0	26.0	42.5	18.0	4.5
17	Pennsylvania	Iowa	35	16	16	Bl.	4,800,000	90	6,500	57.0	4.5	3.0	30.0	5.0	0.5	11.0	21.0	39.0	22.0	7.0
18	Ohio	Ohio	45	16	48	Br.	4,600,000	90	6,900	65.5	5.5	1.5	25.5	1.0	1.0	16.0	35.0	29.0	18.0	2.0
19	Wisconsin	Wisconsin	45	16	82	Br.	4,800,000	92	7,300	68.5	4.0	2.0	23.0	2.0	0.5	14.0	30.0	42.0	13.0	1.0
20	Europe	Europe	24	16	16	Bl.	4,640,000	95	7,500	61.0	4.0	1.5	31.0	0.5	2.0	13.0	35.0	33.0	16.0	3.0
21	Illinois	Illinois	29	16	16	Br.	4,900,000	92	7,200	70.0	4.5	2.0	23.0	0.5	0.0	24.0	29.0	34.0	13.0	0.0
22	Michigan	Michigan	24	16	16	Bl.	4,800,000	90	6,100	53.0	5.0	1.0	38.5	1.5	1.0	22.0	34.0	29.0	14.0	1.0

[a] Bl. means blond, Br. means brunette, M. means mixed type of complexion.

TABLE V.—*Differential and Arneth counts on the blood of Americans residing in the Philippines*—Continued.

No.	Birthplace.	Residence.	Age.	Tropical service, months.		Complexion.[a]	Red cells.	Hb.	White cells.	Polynuclears.	Large lymphocytes.	Transitionals.	Small lymphocytes.	Eosinophiles.	Mast cell.	Arneth classification.				
				Present tour.	Total.											I.	II.	III.	IV.	V.
								P. ct.												
23	Rhode Island	Massachusetts	27	16	16	Br.	4,800,000	92	10,000	59.5	4.5	1.0	28.0	5.5	1.5	26.0	39.0	25.0	8.0	2.0
24	Illinois	Illinois	22	16	16	Bl.	4,800,000	80	7,700	49.5	5.5	1.5	31.0	12.5	0.0	16.0	27.0	38.0	18.0	1.0
25	Missouri	Missouri	27	16	16	Bl.	4,200,000	85	7,500	63.4	4.1	1.5	29.5	0.5	1.0	17.0	32.0	33.0	16.0	2.0
26	Kentucky	Kentucky	20	16	16	Br.	4,640,000	90	7,800	58.0	7.0	3.0	31.5	0.5	0.0	13.0	31.0	37.0	18.0	1.0
27	New York	New York	28	16	16	Br.	4,440,000	82	7,900	57.5	5.5	1.0	30.0	6.0	0.0	14.0	36.0	32.0	16.0	2.0
28	Michigan	Colorado	23	16	16	Bl.	4,560,000	85	7,300	69.0	5.5	2.5	19.0	3.5	0.5	10.0	36.0	36.0	17.0	1.0
29	Indiana	Indiana	34	16	18	Br.	4,200,000	80	7,600	67.0	5.0	1.5	24.5	1.5	0.5	13.0	24.0	45.0	16.0	2.0
30	Massachusetts	Massachusetts	30	17	17	Br.	4,640,000	85	7,400	57.5	4.5	2.0	34.5	1.5	0.0	12.0	47.0	32.0	9.0	0.0
31	Tennessee	Washington	22	16	16	Bl.	4,460,000	85	6,700	43.5	9.5	2.0	40.5	3.5	1.0	6.0	31.0	42.0	18.0	3.0
32	Europe	Iowa	28	16	43	Bl.	4,200,000	90	5,000	47.0	3.5	1.0	48.5	0.0	0.0	4.0	24.0	43.0	27.0	2.0
33	Massachusetts	Massachusetts	29	16	61	Bl.	2,490,000	80	7,400	56.0	7.0	1.5	32.5	2.5	0.5	11.0	21.0	33.0	28.0	7.0
34	Europe	Europe	23	16	16	Br.	4,960,000	95	7,900	58.5	6.0	0.5	33.0	1.5	0.5	10.0	36.0	36.0	14.0	4.0
35	Europe	Utah	23	16	16	Br.	5,200,000	90	6,500	60.5	6.0	1.5	27.0	4.5	0.5	7.0	27.0	28.0	33.0	5.0
36	Europe	South Dakota	23	16	16	Bl.	4,440,000	80	8,500	45.0	5.5	0.5	39.0	8.0	2.0	23.0	23.0	35.0	17.0	2.0
37	Europe	Europe	27	16	16	Bl.	4,300,000	88	7,000	50.0	4.5	1.0	33.5	10.5	0.5	7.0	30.0	36.0	24.0	3.0
38	Europe	Minnesota	36	16	52	Bl.	4,840,000	92	8,300	64.5	5.0	1.0	26.0	2.5	1.0	9.0	29.0	41.0	20.0	1.0
39	Massachusetts	Massachusetts	30	24	42	Bl.	4,320,000	85	4,200	35.5	4.0	1.5	50.5	8.5	0.0	9.0	35.0	40.0	13.0	3.0
40	District of Columbia.	Arizona	18	16	16	Bl.	5,000,000	85	7,700	42.0	3.0	2.5	49.5	2.0	1.0	15.0	34.0	40.0	11.0	0.0
41	New York	New York	34	16	42	Br.	4,760,000	85	10,700	66.5	2.5	1.5	29.0	0.5	0.0	41.0	44.0	12.0	3.0	0.0
42	California	Missouri	22	16	16	Bl.	4,452,000	87	8,200	71.5	1.5	0.5	22.0	2.5	2.0	9.0	40.0	29.0	19.0	3.0
43	California	California	23	24	24	Br.	5,000,000	90	5,900	60.0	4.0	1.5	29.0	4.5	1.0	6.0	27.0	46.0	19.0	2.0
44	Texas	Texas	36	16	40	Br.	4,690,000	93	7,500	63.5	5.5	1.5	28.0	0.5	1.0	12.0	37.0	39.0	10.0	2.0
45	North Dakota	North Dakota	27	24	24	Br.	5,200,000	95	7,300	50.0	3.0	1.5	38.0	7.0	0.5	8.0	40.0	38.0	13.0	1.0

[a] Bl. means blond, Br. means brunette, M. means mixed type of complexion.

46	Missouri	Oklahoma	38	16	64	Br.	4,710,000	90	7,100	46.0	7.0	1.0	39.0	6.0	1.0	8.0	42.0	32.0	14.0	4.0
47	Europe	Europe	21	16	16	Bl.	4,480,000	84	6,200	54.5	7.5	1.0	34.5	2.0	0.5	4.0	29.0	42.0	23.0	2.0
48	Europe	Europe	39	16	41	Br.	4,840,000	95	9,000	65.5	5.0	1.5	22.0	3.0	3.0	9.0	33.9	37.0	16.0	5.0
49	Missouri	Oklahoma	30	16	76	Bl.	4,600,000	92	8,600	61.0	3.5	0.5	27.0	7.5	0.5	21.0	48.0	26.0	5.0	0.0
50	Europe	Europe	29	16	40	Br.	4,640,000	90	5,500	66.5	5.5	0.0	24.5	3.0	0.5	8.0	38.0	40.0	12.0	2.0
51	New York	New York	30	6	38	Br.	----------	-----	-------	62.0	8.0	4.0	23.0	2.0	1.0	3.0	20.0	48.0	23.0	6.0
52	Canada	Canada	40	15	51	Bl.	----------	-----	-------	62.0	13.0	5.0	17.0	3.0	0.0	10.0	34.0	39.0	15.0	2.0
53	Missouri	Missouri	19	6	6	M.	----------	-----	-------	66.0	6.5	1.5	25.5	0.5	0.0	5.0	16.0	50.0	24.0	5.0
54	Illinois	Illinois	28	7	7	M.	----------	-----	-------	54.0	2.0	8.0	26.0	8.0	2.0	8.0	28.0	45.0	17.0	2.0
55	Virginia	Virginia	31	4	18	Br.	----------	-----	-------	58.5	5.5	2.5	29.0	3.0	1.5	26.0	45.0	23.0	5.0	1.0
56	Texas	Texas	24	6	18	Br.	----------	-----	-------	60.0	2.0	2.0	29.0	5.5	1.5	12.0	37.0	35.0	15.0	1.0
57	Michigan	Michigan	29	14	74	Br.	----------	-----	-------	68.5	5.0	2.0	23.5	0.0	1.0	16.0	46.0	30.0	7.0	1.0
58	New York	New York	23	22	22	Bl.	----------	-----	-------	54.5	3.0	3.5	34.5	3.0	1.5	6.0	42.0	41.0	9.0	2.0
59	Arkansas	Arkansas	25	8	8	Br.	----------	-----	-------	30.0	3.0	3.0	42.5	21.5	0.0	18.0	39.0	37.0	6.0	0.0
60	United States	United States	40	12	12	M.	----------	-----	-------	43.5	11.0	5.0	36.0	3.0	1.5	16.0	38.0	35.0	9.0	2.0
61	Europe	Pennsylvania	25	15	34	Br.	5,000,000	80	8,000	61.0	3.0	2.0	30.5	3.5	0.0	17.0	40.0	32.0	11.0	0.0
62	Europe	New York	25	15	34	Br.	5,800,000	85	7,200	58.0	1.5	7.0	31.5	2.0	0.0	15.0	24.0	41.0	17.0	3.0
63	Pennsylvania	Pennsylvania	23	16	36	Br.	5,600,000	80	7,800	50.0	3.0	3.5	39.5	3.5	0.5	16.0	39.0	30.0	13.0	2.0
64	Pennsylvania	Pennsylvania	27	16	36	Br.	5,200,000	95	6,000	46.5	3.5	3.5	39.5	6.5	0.5	18.0	31.0	43.0	8.0	0.0
65	Virginia	Virginia	27	16	36	Br.	4,400,000	80	8,000	58.0	2.0	4.0	32.5	2.5	1.0	12.0	40.0	33.0	14.0	1.0
66	Illinois	New York	28	15	34	Bl.	6,000,000	85	7,200	68.5	2.0	1.5	18.0	9.0	1.0	14.0	51.0	32.0	2.0	1.0
67	Indiana	Indiana	24	15	31	Bl.	4,800,000	100	5,600	56.0	2.0	2.0	33.5	6.0	0.5	5.0	27.0	51.0	15.0	2.0
68	New York	New York	25	16	16	Bl.	5,200,000	80	7,000	60.0	1.0	1.5	33.5	3.5	0.5	12.0	45.0	40.0	3.0	0.0
69	Iowa	Iowa	20	15	31	Bl.	4,800,000	85	7,200	50.0	1.5	5.0	41.5	1.0	1.0	16.0	37.0	38.0	9.0	0.0
70	Ohio	United States	30	15	64	Bl.	5,200,000	90	7,800	50.0	5.0	1.5	35.0	7.0	1.5	17.0	30.0	44.0	9.0	0.0
71	Kentucky	Texas	38	13	97	Br.	6,440,000	90	9,100	56.0	2.5	3.0	24.0	13.5	1.0	17.0	31.0	45.0	7.0	0.0
72	Europe	New York	42	13	76	Br.	5,336,000	85	5,900	69.0	4.0	5.0	15.0	4.0	3.0	16.0	26.0	46.0	10.0	2.0
	Average		----	14.6	28.7	-------	4,767,000	89	7,304	56.8	4.7	2.2	31.7	3.8	0.8	13.3	32.9	37.2	14.6	2.0

TABLE VI.—*Differential and Arneth counts on the blood of Filipinos.*

Number.	Age.	Hb.	White cells.	Polynuclears.	Small lymphocytes.	Large lymphocytes.	Transitional.	Eosinophiles.	Mast cell.	Arneth classification.				
										I.	II.	III.	IV.	V.
		P. ct.												
1	17	90	8,900	51.5	36.5	4.5	1.5	5.0	1.0	14	38	36	10	2
2	22	90	9,600	57.0	21.5	5.5	1.5	14.0	0.5	21	30	31	15	3
3	19	94	9,700	52.5	33.5	3.5	0.5	9.5	0.5	29	32	31	6	2
4	29	92	9,800	64.5	25.5	1.5	2.0	6.0	0.5	30	32	29	9	0
5	24	85	10,800	75.5	12.5	2.0	2.5	7.5	0.0	24	35	30	10	1
6	30	87	7,900	72.0	15.5	1.5	1.0	10.0	0.0	20	33	28	16	3
7	33	95	12,800	58.5	32.5	3.5	1.0	3.0	1.5	33	32	26	6	3
8	23	93	9,900	48.5	31.0	4.0	0.5	15.5	0.5	39	38	14	7	2
9	24	85	7,500	66.5	24.0	5.0	2.5	2.0	0.0	21	35	37	5	2
10	37	86	10,600	50.5	34.5	5.5	2.0	6.5	1.0	20	31	31	16	2
11	23	95	11,000	63.0	27.5	1.0	0.0	7.0	1.5	30	31	25	14	0
12	30	88	10,400	46.0	31.5	3.0	1.0	17.5	1.0	35	44	15	6	0
13	17	95	11,500	61.0	26.5	2.5	1.0	7.0	2.0	17	39	33	10	1
14	27	94	8,600	44.0	43.5	5.0	1.5	5.0	1.0	22	44	23	10	1
15	38	96	11,200	62.5	23.5	6.5	0.5	6.5	0.5	33	36	21	10	0
16	32	98	8,000	42.0	42.0	5.0	0.5	10.0	0.5	24	34	32	10	0
17	30	99	8,400	55.0	30.0	4.0	1.0	9.0	1.0	35	40	22	3	0
18	36	90	7,300	60.0	27.5	5.5	1.5	5.0	0.5	23	41	30	6	0
19	36	93	9,300	67.5	25.5	2.0	1.0	2.5	1.5	33	33	28	5	1
20	24	95	8,400	51.5	30.5	2.5	0.0	15.5	0.0	22	41	28	9	0
21	17	92	11,100	41.5	39.5	6.5	1.5	10.0	1.0	22	25	32	19	2
22	25	100	8,700	46.0	43.0	2.5	0.0	8.5	0.0	33	36	23	8	0
23	22	90	8,300	33.0	52.0	0.5	1.0	13.0	0.5	21	33	35	9	2
24	24	93	5,500	50.0	38.0	2.0	1.0	9.0	0.0	42	42	12	4	0
25	36	99	7,900	42.5	38.0	5.0	1.5	12.5	0.5	42	38	15	5	0
26	36	97	9,900	60.5	28.0	2.0	0.5	9.0	0.0	29	43	20	8	0
27	22	98	10,100	41.0	42.0	0.5	0.5	16.0	0.0	24	37	29	9	1
28	43	99	6,900	61.5	25.5	3.5	1.5	5.0	0.0	14	35	36	14	1
29	45	94	8,200	70.5	23.5	1.5	0.5	3.5	0.5	32	44	18	6	0
30	-----	------	--------	50.0	33.0	3.5	6.0	7.0	0.5	28	40	30	1	1
31	-----	------	--------	59.5	17.0	3.5	3.5	14.5	2.0	24	46	28	2	0
32	-----	------	--------	59.0	26.5	5.0	2.0	6.5	1.0	28	45	21	5	1
33	-----	------	--------	47.5	33.5	3.5	3.5	11.5	0.5	25	40	31	4	0
34	-----	------	--------	47.0	32.5	7.0	6.0	7.0	0.5	35	38	16	10	1
35	-----	------	--------	33.5	47.0	2.5	1.0	15.5	0.5	36	34	24	6	0
36	-----	------	--------	37.5	49.0	4.0	3.0	6.5	0.0	28	46	19	6	1
37	-----	------	--------	54.0	17.0	11.0	9.0	9.0	0.0	31	47	22	0	0
38	-----	------	--------	52.5	8.5	10.0	2.0	26.5	0.5	27	37	30	6	0
39	-----	------	--------	49.0	29.5	5.5	1.5	13.0	1.5	27	46	23	4	0
40	-----	------	--------	54.0	15.5	18.5	4.0	6.5	1.5	16	37	35	12	0
41	-----	------	--------	50.0	11.0	8.5	8.5	21.0	1.0	14	39	28	16	3
42	-----	------	--------	24.5	27.0	5.5	2.0	38.5	2.5	34	46	20	0	0
43	-----	------	--------	43.5	45.5	5.5	2.5	1.5	1.5	54	41	5	0	0
44	-----	------	--------	50.0	32.0	5.0	1.0	11.5	0.5	40	47	13	0	0
45	-----	------	--------	42.0	33.0	6.0	7.0	11.5	0.5	30	43	22	4	1
46	-----	------	--------	48.5	34.0	4.0	1.0	12.5	0.0	8	33	37	19	3
47	-----	------	--------	69.5	14.5	2.5	1.0	11.5	1.0	33	44	16	7	0
48	-----	------	--------	63.5	22.5	4.5	2.0	6.5	1.0	38	39	22	1	0
49	-----	------	--------	35.5	25.5	10.0	0.0	29.0	0.0	13	39	44	4	0
50	-----	------	--------	39.5	37.0	5.5	0.0	17.5	0.5	21	39	33	4	3
Average		93	9,248	52.2	29.9	4.6	2.0	10.6	0.7	27.5	38.3	25.8	7.5	0.9

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THE SO-CALLED X-BODIES AS ARTEFACTS IN GLASS SLIDES.[1]

By WESTON P. CHAMBERLAIN and EDWARD B. VEDDER.[2]

(From the United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands.)

Dr. Andrew Balfour(1) has announced the finding of the so-called X-bodies in the blood of a patient suffering from urticaria. These bodies were first reported by Horrocks and Howell(2) who found them in a case of tertian malaria, in several cases of undetermined fever, in the citrated blood from a rabbit and in the blood of oxen. They describe the X-bodies as follows:

> "The bodies, when stained, were characterized by a faint capsule with a circular centre staining deep blue; they varied in size, some being as large as a red corpuscle, others only about one-eighth the size of a red corpuscle. In addition to these forms, which were the most common, the following were also seen: (*a*) A small blue circular centre surrounded by four or more faint capsules, concentrically arranged; (*b*) two circular bodies, each having a dark blue central point surrounded by a light blue ring, enveloped in one capsule which appeared indented as if two capsules were in the process of formation; (*c*) similar to (*b*) but the part surrounding the deep blue centre stained a deeper blue, and two indented capsules were seen; (*d*) a dark blue central part shaped like a crescent, containing a small circular body, with a deep blue central point within the arms of the crescent. None of the bodies on the slide showed any signs of chromatin."

Neither Horrocks and Howell nor Balfour commit themselves as to the nature of these bodies. Indeed, the former authors say, "Having in view the diverse nature of the cases in which the X-bodies were found, we are not justified in considering that they were the cause of the pathological conditions observed." It occurred to Balfour that such bodies might be contaminations from the skin, but this idea was subsequently dismissed, and none of these investigators appear to doubt that the bodies were really obtained from the blood. Fortunately they both furnish good and detailed drawings which permit of easy identification of the X-bodies.

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[1] Published by permission of the Chief Surgeon, Philippines Division.

[2] Weston P. Chamberlain, major, Medical Corps, United States Army and Edward B. Vedder, captain, Medical Corps, United States Army, members of the United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands.

We believe that we can point out the nature of these bodies, and that we should do so, not only because two papers have been published on the subject, but because it has come to our knowledge that other investigators have been misled by them. Therefore, a statement of their true nature may simplify investigations in the future.

These peculiar bodies were first observed by one of us in 1907 while studying the blood of dengue, and before the publication of the paper by Horrocks and Howell. They were at first thought to be parasites associated with that disease, but further study showed that they were frequently present in normal blood. Subsequently they were found again in specimens from various pathologic conditions, and from different parts of the body, and the idea was finally suggested that they were artefacts in the glass slides. Search of a number of plain unstained glass slides having no specimens on them revealed the fact that these bodies could be found on many. On such unstained slides the bodies have the same morphology, but are without coloration. When the glass slide is previously stained with a little Wright's stain, as though staining a blood film, the bodies take on exactly the appearance described and accurately depicted by Horrocks and Howell and by Balfour.

The X-bodies in these two papers are, therefore, artefacts of some sort, present in certain of the glass slides for microscopic use. We think that they are found more often in old slides which have been used several times, and have been kept for some time in the Tropics. We believe that anyone finding the X-bodies in a preparation can readily verify our statement by staining and examining some of his slides on which no smear has been made. The bodies are not in the stain, because they may be found in unstained preparations.

Other facts that confirm these observations are: 1. The X-bodies have been found on slides made under a great diversity of conditions from both men and animals, including the blood from cases of malaria, undetermined fever, urticaria and dengue, and normal blood in the human, and also the blood of oxen and rabbits and the liver of oxen. We have observed them in slides containing scrapings of skin mounted in liquor potassæ. 2. In the same case the bodies are not found constantly, but are only seen in a few out of many smears, although they are usually numerous in the particular slides in which they are observed. If present in the circulating blood, they should be found on all or nearly all the slides examined from the case at the same time, though not necessarily in large numbers. 3. So far as we are aware the bodies have never been detected anywhere except in smears or preparations made on glass slides.

As further evidence in favor of the X-bodies being in the surface of the slide we performed the following experiment:

A blood smear was stained with Wright's stain and showed many brightly colored X-bodies of various sizes. Several large ones were located with the vernier scale of the mechanical stage. The slide was then removed and scrubbed with water and gauze. On again bringing the located areas under the lens no blood corpuscles could be seen but the X-bodies were still there and unchanged in appearance except that the coloration was less intense. The slide was again removed, scrubbed with alcohol, and replaced beneath the lens. The identical X-bodies were present in the same locations as before, but all trace of the stain had been removed from them.

If a clean new slide containing no specimen is stained in the usual manner with Wright's stain, and the stain-film is then rubbed off with dry gauze or with xylol, X-bodies, still showing coloration, may be found. This fact suggests that the objects named X-bodies in most instances may be due to minute portions of the stain being retained mechanically in microscopic pits in the glass. Usually, the X-bodies are seen to be at a lower level than the blood corpuscles on a slide. We rarely have found instances where they appeared to be above the corpuscles and the edge seemed to overlap a red cell. Such an appearance could hardly have been due to a pit, but might be caused by a minute scale of glass on the surface of the slide, which would entangle the stain and beneath which the edge of a corpuscle could slip. The slide on which this overlapping was noted is the one described in the preceding paragraph and the X-bodies which appeared to have the edges slightly overlying red cells were the ones which remained unchanged in location after repeated scrubbing.

Photomicrographs of these bodies are appended. The photographs were made by Mr. Charles Martin of the Bureau of Science, to whom we desire to express our obligation. The magnification in all cases is 1,000 diameters. The first three figures are photographs of X-bodies found on a slide containing a smear of normal human blood, while the last three are of bodies that were found on a glass slide containing no specimen of any kind, but which had been stained with Wright's stain and the dry stain-film then rubbed off with a piece of gauze. The bodies were very numerous on these slides, a number being present in nearly every field, but it is almost impossible to procure good photographs of more than one or two on a single negative owing to the difficulty of obtaining an accurate focus on several of them at the same time.

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ILLUSTRATIONS.

PLATE I.

FIGS. 1, 2, and 3. X-bodies on slides smeared with normal blood. × 1,000.

FIGS. 4, 5, and 6. X-bodies on a clean slide which had been stained with Wright's stain and the stain-film rubbed off with gauze. × 1,000.

Photomicrographs by Charles Martin of the Bureau of Science, Manila.

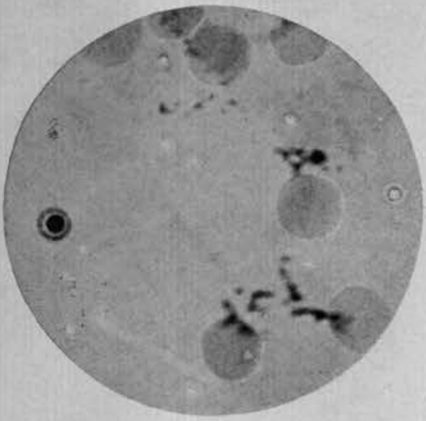

Fig. 1.

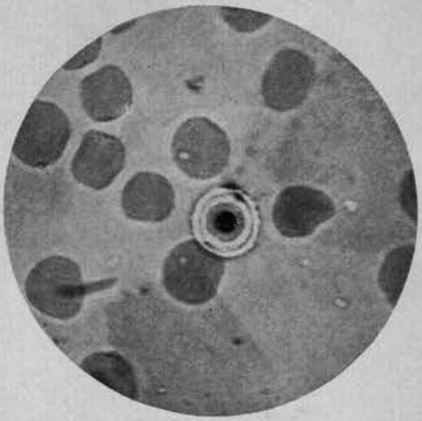

Fig. 2.

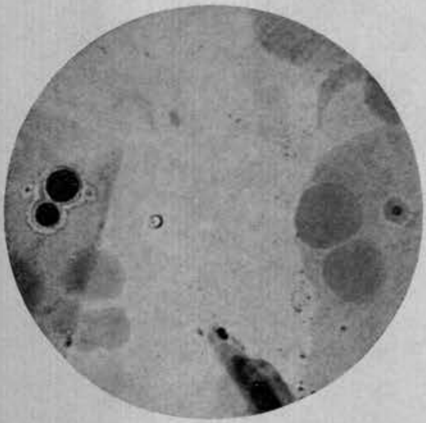

Fig. 3.

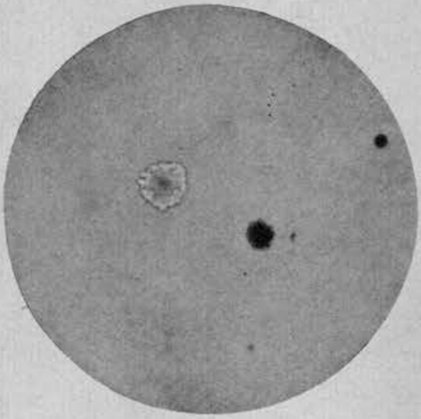

Fig. 4.

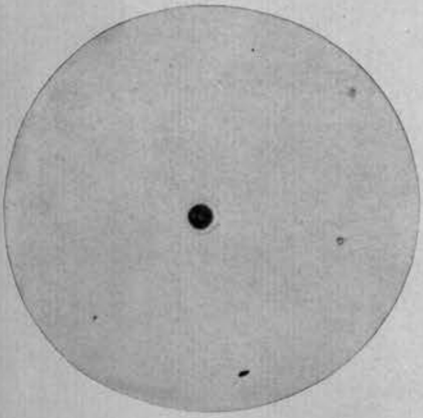

Fig. 5.

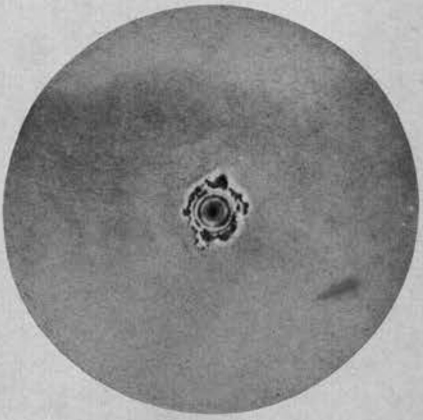

Fig 6.

PLATE I.

Order No. 413.

A MANUAL OF PHILIPPINE SILK CULTURE.

By Charles S. Banks.

(Publication of the Bureau of Science.)

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